Novel, Potent Aldose Reductase Inhibitors: 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic Acid (Zopolrestat) and Congeners

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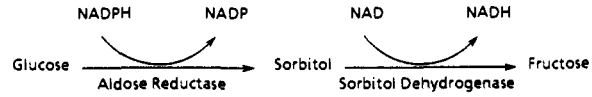
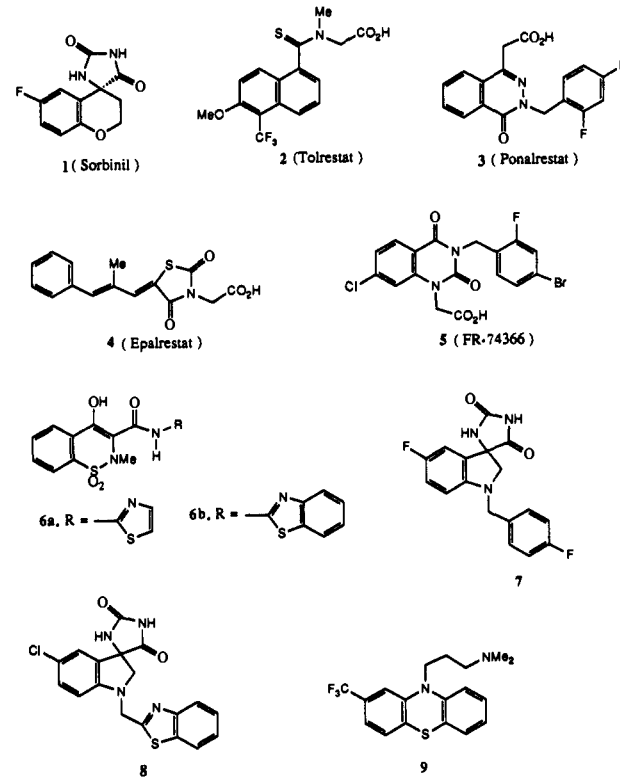
A new working hypothesis that there is a hitherto unrecognized binding site on the aldose reductase (AR) enzyme with strong affinity for benzothiazoles was pursued for the design of novel, potent aldose reductase inhibitors (ARIs). The first application of this hypothesis led to a novel series of 3,4-dihydro-4-oxo-3-(benzothiazolylmethyl)-1-phthalazineacetic acids. The parent of this series (**207**) was a potent inhibitor of AR from human placenta (IC_{50} = 1.9 × 10^{-8} M) and was orally active in preventing sorbitol accumulation in rat sciatic nerve, in an acute test of diabetic complications (ED_{50} = 18.5 mg/kg). Optimization of this lead through medicinal chemical rationale, including analogy from other drug series, led to more potent congeners of **207** and culminated in the design of 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (**216**, CP-73,850, zopolrestat). Zopolrestat was found to be more potent than **207**, both in vitro and in vivo. Its IC_{50} against AR and ED_{50} in the acute test were 3.1 × 10^{-9} M and 3.6 mg/kg, respectively. Its ED_{50}s in reversing already elevated sorbitol accumulation in rat sciatic nerve, retina, and lens in a chronic test were 1.9, 17.6, and 18.4 mg/kg, respectively. It was well absorbed in diabetic patients, resulting in high blood level, showed a highly favorable plasma half-life (27.5 h), and is undergoing further clinical evaluation. An assortment of synthetic methods used for the construction of benzothiazoles, including an efficient synthesis of zopolrestat, is described. Structure-activity relationships in the new series are discussed.

Chronic diabetes leads to long-term complications which include neuropathy, nephropathy, retinopathy, and cataracts. A hallmark of diabetes is hyperglycemia, and at the biochemical level, this results in the intracellular production of excess sorbitol and fructose due to an increased glucose flux through the so-called sorbitol pathway, also known as the polyol pathway.[1] This pathway is regulated



by two enzymes, aldose reductase (AR) and sorbitol dehydrogenase. AR is present in all tissues susceptible to diabetic complications. In nerve, lens, and retina, where insulin is not necessary for glucose transport across the cell membrane, the glucose concentration inside the cell can be equal to the plasma glucose concentration. The high cellular glucose is metabolized by these tissues to sorbitol, which accumulates in a higher than normal concentration because it does not readily cross the cell membrane and is only slowly metabolized to fructose. Available pharmacological and emerging clinical data with extant ARIs strongly suggest that diabetic complications could be a consequence of increased flux through the polyol pathway and/or high intracellular accumulation of sorbitol.[2] Spurred by this, several pharmaceutical companies are continuing their efforts to develop ARIs for clinical evaluation. Our objective was to discover a safe and sufficiently potent ARI with potential for once-a-day therapy of diabetic complications.

There are, to date, two major structural classes of aldose reductase inhibitors—spirohydantoins and carboxylic acids.[3,4] Several spirohydantoins[5] including the first of its class, sorbinil, show excellent activity in both in vitro and in vivo models of diabetic complications. Encouraging clinical results have been reported with sorbinil in the treatment of diabetic painful neuropathy.[6] However, a low incidence of hypersensitivity side effect with sorbinil shifted our attention to the discovery of potent nonhydantoin ARIs. A variety of carboxylic acids, including NSAIDs,[3,4] are known to show good to excellent AR inhibitory activity in vitro. However, to date only four clinical candidates—tolrestat,[7] ponalrestat,[8] epalrestat,[9]



† Department of Medicinal Chemistry.
‡ Department of Metabolic Diseases.

(1) For recent reviews, see (a) Kador, P. F.; Robinson, W. G.; Kinoshita, J. H. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 691. (b) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.

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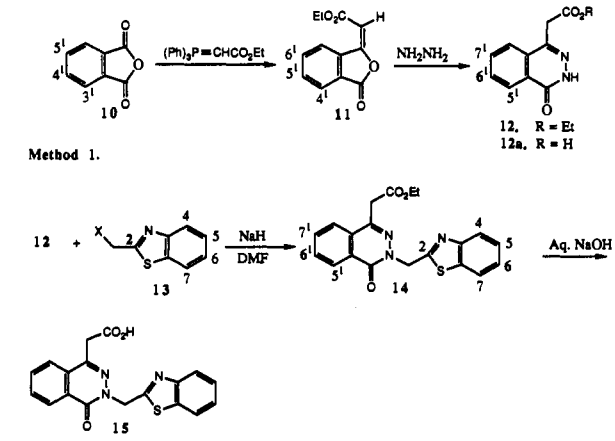
and **5** (FR-74360)[10]—have emerged. In our hands, ponalrestat (**3**, 3,4-dihydro-4-oxo-3-(2-fluoro-4-bromobenzyl)-1-phthalazineacetic acid) was the most potent compound among the first three that were tested in animal models of diabetic complications. Because of the relative paucity of carboxylic acid structures with potent in vivo activity, we were attracted to explore the possibility of elaborating the 3,4-dihydro-4-oxo-1-phthalazineacetic acid backbone to obtain novel, potent ARIs. However, we could not discern any clues from the extensive SAR work of Brittain and co-workers on ponalrestat to guide our efforts.[8]

At this time, in our own ARI program, we made the observation that certain other heterocyclic backbones with a pendent benzothiazole group showed good ARI activity. One of the early observations is illustrated for two members of the oxicam family of compounds—**6a** (inactive at 10^{-4} M) and **6b** ($IC_{50} = 5.5 \times 10^{-6}$ M). This large increase in potency in going from a thiazole to a benzothiazole side chain and additional observations relating to the role of benzothiazole in other series of compounds (cf. **7** and **8**; IC_{50}s 1.4×10^{-7} and 3.7×10^{-8} M, respectively) led us to propose a working hypothesis that there is a hitherto unrecognized binding site on the AR enzyme with strong affinity for benzothiazoles at some distance from a site which binds to acidic groups. We now report the first application of this hypothesis in the carboxylic acid area which has yielded a novel series of phthalazinoneacetic acid ARIs and has led to the discovery of our clinical candidate, 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (**216**, zopolrestat).

Chemistry

Ethyl 3,4-dihydro-4-oxo-1-phthalazineacetate (**12**) was prepared by reacting (*E*)-3-[(ethoxycarbonyl)-methylidene]phthalide[11a] (**11**), or the corresponding *Z* isomer,[11b] with hydrazine (Scheme I). Congeners of **12** with substituents on the benzene ring were also prepared according to the above procedure and are shown in Table I. Wittig olefination of 3′-substituted phthalic anhydrides gave exclusively 4′-substituted methylidenephthalide esters (cf. **11**), which when reacted with hydrazine resulted in 5′-substituted phthalazinone esters. When 4′-substituted phthalic anhydrides were used, a mixture of 5′- and 6′-phthalides was obtained. This mixture was used directly in the next step to obtain a mixture of 6′- and 7′-phthalazinone esters, which was separated into its components by chromatography.

Scheme I



Scheme II

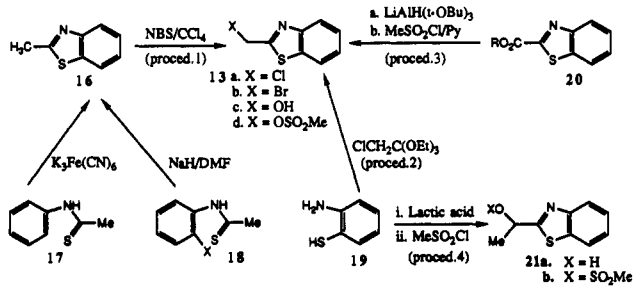
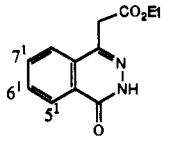


Table I. Physical Constants of Benzo-Substituted Ethyl 3,4-Dihydro-4-oxo-1-phthalazineacetates



compd	subst[a]	mp,[b] °C	compd	subst[a]	mp,[b] °C
12	none[c]		**62**	2-Me	
54	5′-F[c]		**63**	6′-Cl	
55	5′-Me[c]		**64**	6′-Br	
56	7′-Cl	250	**65**	7′-Br	
57	6′,7′-Cl_2	224–225	**66**	6′-OMe	
58	6′-NO_2	228–230	**67**	6′-CF_3	*d*
59	7′-CH_3	231–232	**68**	7′-CF_3	*d*
60	7′-OCH_3	172–174	**69**	6′-*i*Pr	*d*
61	7′-NO_2	167–169	**70**	7′-*i*Pr	*d*

[a] Structures of all new compounds were confirmed by NMR and MS. [b] Melting points for compounds **62** → **70** were not determined. [c] Brittain, D. R.; Wood, R. U.S. Patent 4,251,528, Feb. 17, 1981. [d] NMR spectra for these compounds are reported in EP 222,576, 1987 (*Chem. Abstr.* 1987, *107*, 176055).

Four methods were used to prepare precursor esters **14** of target carboxylic acids **15**. Method 1 consisted of alkylating esters **12a**, with appropriately substituted 2-(halomethyl)benzothiazoles (Table II), in the presence of sodium hydride or potassium *tert*-butoxide with DMF as solvent. Synthetic routes employed for the preparation of 2-(halomethyl)benzothiazoles are shown in Scheme II. Physical constants of the 2-(halomethyl)benzothiazoles prepared are listed in Table II. 2-(Bromomethyl)benzothiazoles (**13**, X = Br) were prepared by exposure of 2-methylbenzothiazoles (**16**) to *N*-bromosuccinimide under standard conditions. 2-Methylbenzothiazoles were prepared by oxidative cyclization of thioacetanilides[12] or base-induced intramolecular displacement of 2-bromo-

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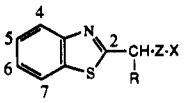
Table II. Physical Constants of 2-(Halomethyl)benzothiazoles

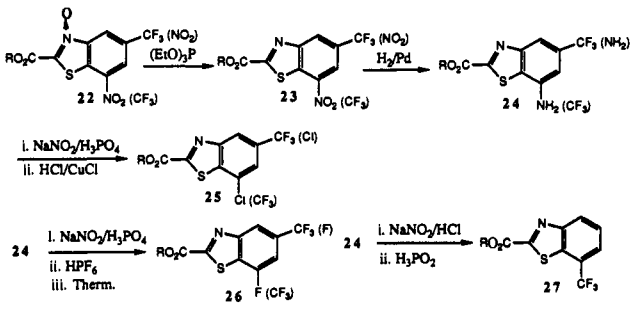


compd	subst	R	Z	X	procedure[a]	mp, °C
71	H[b]	H	–	Cl	2	
72	4-F[b]	H	–	Cl	2	*c*
73	5-F[b]	H	–	Cl	2	
74	4-Cl	H	–	Cl	2	114–115
75	5-Cl	H	–	Cl	2	78–80
76	6-Cl[d]	H	–	Br	1	
77	5-Br	H	–	Br	1	106–108
78	6-Br	H	–	Br	2	108
79	7-Br	H	–	Br	1	*c,e*
80	5-CF_3[b]	H	–	Cl	2	
81	6-CF_3[b]	H	–	Cl	2	
82	6-CH_3[d]	H	–	Br	3	53
83	4-OMe[d]	H	–	Cl	2	114–116
84	5-OMe	H	–	Cl	2	*c,f*
85	6-OMe[d]	H	–	Br	1	111
86	6-*i*Pr[d]	H	–	Cl	3	111
87	4,5-F_2	H	–	Cl	2	*c,g*
88	5,7-F_2	H	–	Cl	2	*a*
89	4,5-Cl_2[b]	H	–	Cl	2	68–70
90	4,6-Cl_2	H	–	Br	1	54–55[h]
91	4,7-Cl_2[b]	H	–	Cl	2	*c*
92	5,6-Cl_2[d]	H	–	Br	1	*c*
93	5,7-Cl_2	H	–	Cl	2	
94	5,7-$(OMe)_2$[b]	H	–	Cl	2	105[h]
95	5-F,7-CF_3[d]	H	–	Cl	3	
96	5-Cl,7-CF_3[d]	H	–	Cl	3	
97	6-Me,7-F[d]	H	–	Cl	2	
98	5-CF_3,7-F[d]	H	–	Cl	3	
99	5-CF_3,7-Cl[d]	H	–	Cl	3	
100	4-OMe,5-F	H	–	Cl	2	127
101	H[d]	CH_3	–	Cl	4	*c*
102	5-Cl	CH_3	–	OSO_2Me	4	92–94
103	5-CF_3	CH_3	–	OSO_2Me	4	84
104	H[d]	H	S	Cl	1[i]	*c*
105	7-aza[b]	H		H	2	*c*

[a] See Scheme III. [b] Reference 15. [c] NMR data for these compounds are reported in EP 222,576, 1987 (*Chem. Abstr.* 1987, *107*, 176055). [d] Structure confirmed by NMR and MS. [e] Starting 2-methylbenzothiazole prepared by potassium fericyanide oxidation of thioacetonlide (cf. 17) derived from 3-bromoaniline and separation of 5- and 7-bromo isomers. [f] Starting 2-methylbenzothiazole, ref 14. [g] Starting material commercially available. [h] Melting points for these compounds in ref 15 are in error. [i] NCS was used in place of NBS.

thioacetanilides[13] (cf. **17** and **18**). 2-(Chloromethyl)-benzothiazoles (**13**, X = Cl) were prepared either by condensing 2-aminobenzenethiols (**19**) with 2-chloro-1,1,1-triethoxyethane[14] or by reacting 2-hydroxybenzothiazoles (**13c**, X = OH) with thionyl chloride. 2-(Chloromethyl)-benzothiazoles were also obtained during attempted formation of corresponding mesylates **13d**. The source of 2-hydroxybenzothiazoles was the corresponding ester **20**. 2-(Chloromethyl)benzothiazoles with 5-CF_3/7-F, 7-Cl; 7-CF_3/7-F, 7-Cl; and 7-CF_3 substituents were prepared via a very intriguing benzothiazole *N*-oxide route[15] (Scheme III). 2-(Ethoxycarbonyl)-7-nitro-5-(trifluoromethyl)-benzothiazole and 2-(methoxycarbonyl)-5-nitro-7-(trifluoromethyl)benzothiazole, obtained by reduction of corresponding *N*-oxides with triethyl phosphite, were hydrogenated to yield the corresponding amines, which were diazotized[16] under strong acid conditions. The diazo group was displaced by appropriate halides to obtain **25** or **26** or reduced with hypophosphorous acid to yield **27**. Esters **20** were converted, in a standard sequence of reactions, to

Scheme III



the corresponding 2-(chloromethyl)benzothiazoles. 1-(2-Benzothiazolyl)ethanol and congeners, precursors of alkylating agents, were obtained by cyclocondensation between amino thiols **19** and lactic acid.[17] Mesylates derived from these hydroxybenzothiazoles were used as alkylating agents. 2-Aminobenzenethiols were prepared by literature procedures involving reduction of *o*-nitro disulfides,[18] hy-

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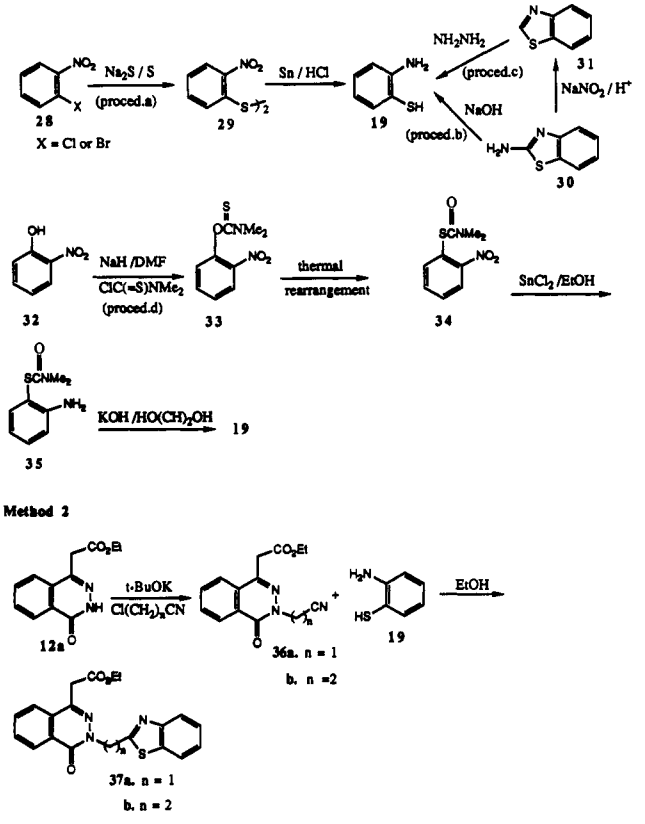
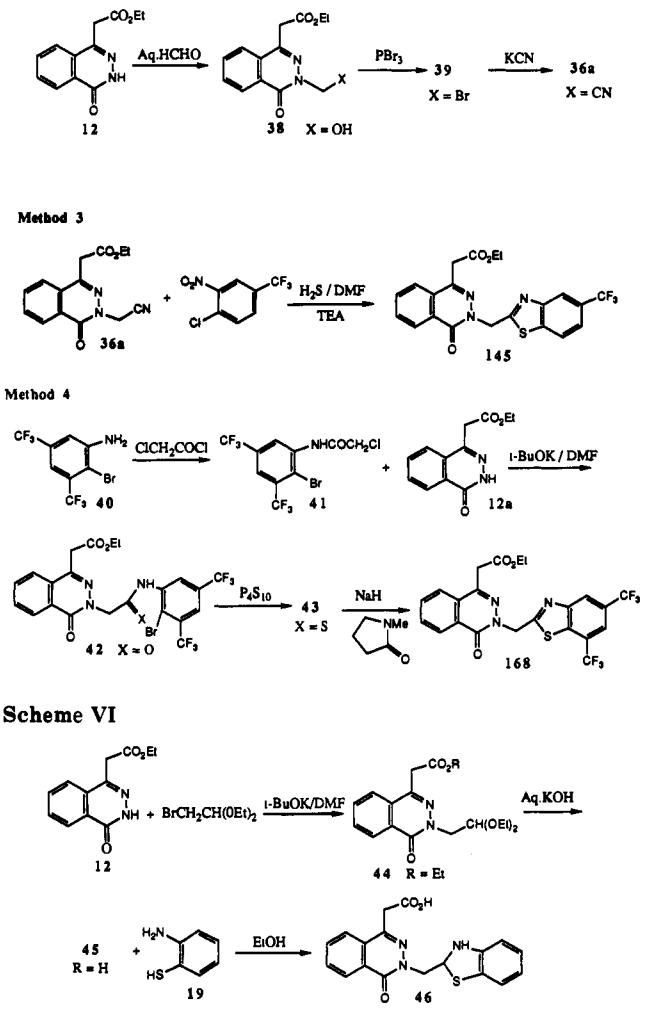
Scheme IV

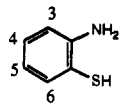


Scheme V



Scheme VI

Table III. Procedures Used for Aminobenzenethiols



no.[a]	subst	procedure,[b] ref	no.[a]	subst	procedure
106	H	commercial	**119**	3,4-F_2	b
107	3-F	b	**120**	4,5-F_2	b
108	4-F	a,20b	**121**	4,6-F_2	b
109	3-Cl	b,20c	**122**	5-Me,6-F	b
110	4-Cl	a,19a	**123**	4-OMe,5-F	b
111	4-Br	a,19b	**124**	3,4-Cl_2	b
112	4-Me	a,19c	**125**	4,6-Cl_2	b
113	3-CF_3	c	**126**	3,6-Cl_2	b
114	4-CF_3	a,19c	**127**	4,6-Me_2	b
115	5-CF_3	c	**128**	4,6-$(OMe)_2$	b
116	3-OMe	b,20c	**129**	4-Cl,6-F	d
117	4-OMe	a,19b	**130**	4-F,6-Cl	d
118	5-*i*Pr	b	**131**	6-aza	c

[a] Structures of all new compounds were confirmed by NMR and MS. [b] See Scheme III. [c] *J. Org. Chem.* **1964**, *24*, 2652.

droxide-mediated ring opening of 2-aminobenzothiazoles[19] (**30**), elaboration of *o*-nitrophenols via Newman procedure,[20] or the rather unusual scission of benzothiazole (**31**) with hydrazine.[21] The last procedure was particularly well-suited for the preparation of 3-CF_3- and 4-CF_3-substituted aminobenzenethiols. The various routes employed are shown in Scheme IV. The list of 2-aminobenzenethiols prepared is shown in Table III.

In method 2, ester **12** was alkylated with chloroacetonitrile and the resulting cyanomethyl compound **36a** was cyclized with 2-aminobenzenethiol hydrochlorides in refluxing ethanol. In an alternative method (Scheme V), the cyano compound **36a** could be prepared by exposure of **12** to aqueous formaldehyde in ethanol to obtain **38** and sequential treatment of **38** with phosphorus tribromide and potassium cyanide. This sequence to **36a** was very valuable in the synthesis of ^{13}C- and ^{14}C-labeled **213** and **216**,[22] due to ready availability of labeled potassium cyanide. Compound **36b** was obtained by following method 2 but with 3-chloropropionitrile in place of chloroacetonitrile.

Method 3 proved to be an extremely efficient route to **216**. For example, exposure of **36a** to 4-chloro-3-nitrobenzotrifluoride in DMF saturated with a continuous stream of hydrogen sulfide gave **145**, precursor of **216**, in high yield.

In method 4 (illustrated for compound **170**), ester **12** was first alkylated with chloroacetanilide **41** to yield **42**, which was thiated with phosphorus pentasulfide to obtain **43**. Intramolecular cyclization of **43** was effected by sodium hydride in hot *N*-methylpyrrolidone.

Esters **205** and **206** were prepared via alkylation of **12** with 3-cyano- and 4-cyanobenzyl bromide, respectively, and then fusion of the resulting substituted benzyl compounds with 2-aminobenzenethiol.

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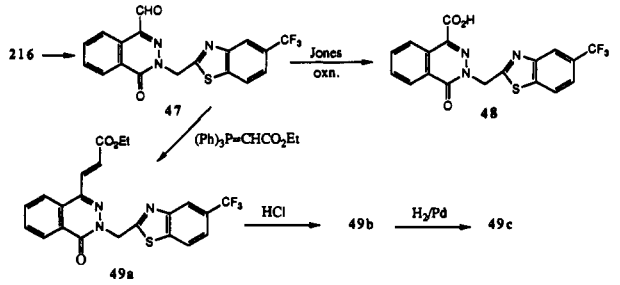
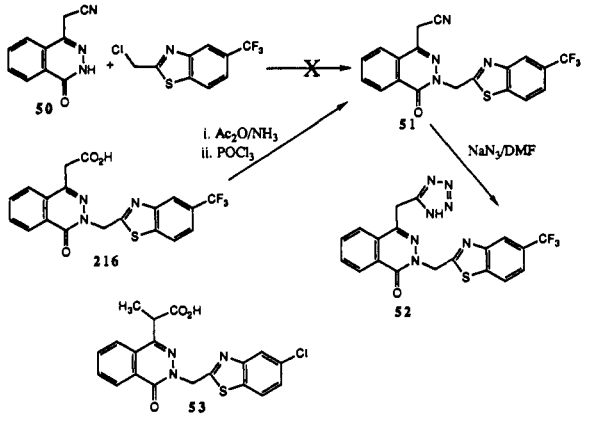
Scheme VII



Scheme VIII



Alkylation of **12** with 2-bromoacetaldehyde diethyl acetal and base hydrolysis of the resulting compound **44**, yielded **45** (Scheme VI). Condensation of **45** with 2-aminobenzenethiol gave 2,3-dihydro analogue **46**. The phenolic acids (e.g. **258**) were prepared by demethylation of the corresponding methoxy compounds in refluxing aqueous hydrobromic acid. The preparation of tetrazole **52** starting from nitrile **50** had to be abandoned because of serious competitive alkylation at carbon α to the nitrile group. Instead, it was prepared from **216** via the corresponding nitrile **51** (Scheme VIII).

Aldehyde **47** was obtained by exposure of **216** to lead tetraacetate. It was subsequently oxidized by Jones procedure to the nor-acid **48**. Wittig olefination of **47** gave ester **49a**. Hydrolysis of **49a** gave the vinylogous acid **49b**, which upon hydrogenation yielded the homologous acid **49c** (Scheme VII).

The precursor esters to target carboxylic acids, prepared in the program, are listed in Table IV. The target carboxylic acids were obtained either by acid or base hydrolysis of the corresponding esters. Analytical data for these acids are compiled in Table V.

Single-crystal X-ray structures of **216**, **246**, **248**, and **254** have been determined.[23] Figure 1 illustrates the overlap of these structures. The orientation of the acetic acid side chain relative to the planar phthalazinone ring is identical with the recorded relationship between the naphthalene ring and acetic acid moieties in naphthalene-1-acetic acid.[24] The conformation of the benzothiazole side chain is nearly the same in all but **246** according to the dihedral angle data in Table VI. In order to discern the difference noted for **246**, MMP_2 molecular energy calculations were done, with a minimization process, to obtain steric energies for the X-ray conformation and its 180° rotated conformation around the C–C bond radiating from the 2-position of the benzothiazole ring.[25] These data, also shown in the Experimental Section, indicate that there is no significant difference in energy for the two conformations in any compound. Therefore, the possibility that **246** could have manifested in a conformation similar to that of the others was confirmed by the single-crystal X-ray data for **288** (chemistry not discussed here), a compound with the same side chain as in **246**, but attached to 8′-azaphthlazinone acetic acid backbone.

Results and Discussion

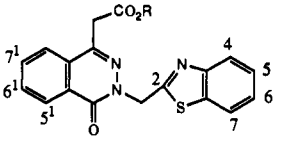
Primary in vitro screening was conducted with AR isolated from human placenta (see the Experimental Section). In vivo, two models based on streptozotocin-induced diabetes in rats were used to evaluate the potential of our compounds in treating diabetic complications. The acute test, a 27-h model with t.i.d. dosing, measured the ability of compounds to prevent sorbitol accumulation in sciatic nerve and lens. The chronic test employing once-a-day dosing for 5 days following 1 week of untreated diabetes assessed the potential of acute test active compounds to normalize already elevated sorbitol levels in the same tissues. Good activity in the chronic test was taken as an encouraging sign that the compound may have potential for once-a-day treatment in the clinic.

Following our hypothesis (vide supra), **207** was prepared and was found to be a potent AR inhibitor in vitro. In the acute rat model, this compound was quite active with an ED_{50} for inhibition of sorbitol accumulation in sciatic nerve of 18.5 mg/kg (vide infra), but it fell short of our goal, which was a compound with a minimum ED_{50} of 5 mg/kg in the chronic test. At this point, it was not clear whether to focus on potential metabolism aspects or on physical chemical properties to improve potency and duration of action.

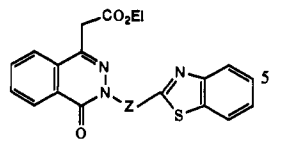
There is very little published information on metabolism of benzothiazole or 2-alkyl benzothiazoles. However, 2-benzothiazolesulfonamide, a potent carbonic anhydrase inhibitor lacking oral diuretic activity, is reported to be metabolized in the dog, to some extent, to 2-mercaptobenzothiazole.[26] Later on, it was found that 2-benzothiazolesulfonamides with 6-OEt (ethoxzolamide), 6-Me, 6-NHCOMe, 4-Me, 4-OMe, and 5-Cl substituents were potent carbonic anhydrase inhibitors but that only the first three manifested oral diuretic activity in the rat.[27] Furthermore, it is known that electrophilic substitution of benzothiazole occurs at the 4- and 6- positions. For nitration, the latter position is preferred by a factor of more than 2.[28] The orientation in electrophilic substitution agrees with the net π-charges estimated by the HMO method.[29] From this knowledge it was reasonable to assume that the 6-position of the benzothiazole side chain in **207** could be susceptible to metabolism, presumably via hydroxylation. Since it was equally reasonable to expect efficient penetration of more lipophilic compounds into target tissues, we embarked on the preparation of 6-substituted congeners with lipophilic substituents. The 6-Cl compound **223** was active both in vitro and in vivo, but was no more potent than the parent, **207**. The more lipophilic 6-Br analogue **224** showed significant potency enhancement in vivo, but 6-*i*Pr, -Me, and -OMe compounds cov-

(23) We thank Dr. J. Bordner of our X-ray Laboratory for these determinations.
(24) Rajan, S. S. *Acta. Crystallogr., Sect. B.* **1978**, *34*, 998.
(25) We thank Dr. B. W. Dominy of our Molecular Modeling Department for calculations and helpful discussion.
(26) Clapf, J. W. *J. Biol. Chem.* **1956**, *223*, 213.
(27) Korman, J. *J. Org. Chem.* **1958**, *23*, 1768.
(28) Ward, E. R.; Poesche, W. H. *J. Chem. Soc.* **1961**, 2825.
(29) *Comprehensive Heterocyclic Chemistry*; Katritzsky, A. R., Rees, C. W., Eds.: Pergamon Press Ltd. **1984**; Vol. 6, p 274.

Table IV. Physical Constants and Method of Preparation of Substituted Phthalazinone Esters



compd	R	subst	method of prep[a]	mp, °C	compd	R	subst	method of prep[a]	mp, °C
132	Me	-	1, 2	143–145	166	Me	5,6-Cl_2	1	*b*
133	Et	4-F	1	119–120	167	Et	5-Cl,6-F	3	202–204
134	Et	5-F	1, 2	118–120	168	Et	5,7-Cl_2	1, 2	144–145
135	Et	4-Cl	1	113–116	169	Et	5,7-Me_2	2	144–147
136	Et	5-Cl	1	152–155	170	Et	5,7-$(CF_3)_2$	1, 4	186–187
137	Me	6-Cl	1	*b*	171	Et	5-CF_3,7-F	1	127–131
138	Et	7-Cl	3	119	172	Et	5-CF_3,7-Cl	1	133–134
139	Et	5-Br	1	159	173	Et	5,7-$(OMe)_2$	1	154–155
140	Et	6-Br	1	*b*	174	Et	5-Cl,7-F	3	202–204
141	Et	7-Br	1	*b*	175	Et	5-F,7-Cl	2	125–126
142	Et	5-Me	1	134–136	176	Et	5-F,7-CF_3	1	*b*
143	Me	6-Me	1	*b*	177	Et	5-Cl,7-CF_3	1	173–174
144	Et	4-CF_3	2	*b*	178	Et	7-aza	1	111–113
145	Et	5-CF_3	1, 2, 3, 4	124–126	179	Et	5′-F	1	
146	Et	6-CF_3	1	138–139	180	Et	6′-Cl	1	
147	Et	7-CF_3	1	*b*	181	Et	7′-Cl	1	
148	Et	4-OMe	1	149–153	182	Et	6′-Br	1	
149	Et	5-OMe	2	127–129	183	Et	7′-Br	1	
150	Me	6-OMe	1	*b*	184	Et	6′-NO_2	1	
151	Et	5-SMe	1	134–136	185	Et	7′-NO_2	1	
152	Et	5-SOMe	*c*	*b*	186	Et	5′-Me	1	
153	Et	5-SO_2Me	*d*	*b*	187	Et	7′-Me	1	
154	Et	6-*i*Pr	1	*b*	188	Et	6′-OMe	1	
155	Et	4,5-F_2	1	118–122	189	Et	7′-OMe	1	
156		4,5-benzo	1	*b*	190	Et	6′-*i*Pr	1	
157	Et	4,7-F_2	2	134–136	191	Et	7-*i*Pr	1	
158	Et	5,6-F_2	2	184	192	Et	6′-CF_3	1	
159	Et	5,7-F_2	1, 2	185	193	Et	7′-CF_3	1	
160	Et	6-Me,7-F	1	136–138	194	Et	6′,7-Cl_2	1	
161	Et	5-OMe,6-F	1	139–140	195	Et	4,5-benzo,5′-F	1	
162	Et	4,5-Cl_2	1	121–122	196	Et	4,5-benzo-7′-Cl	1	
163	Me	4,6-Cl_2	1	*b*	197	Et	4,5-benzo,5′-Me	1	
164	Et	4,7-Cl_2	1	173	198	Et	6-Br,6′-Cl	1	
165	Et	5-CF_3,6-OMe	4	*b*					



compd	Z	subst	mp, °C	compd	Z	subst	mp, °C
199	$-CH_2CH_2-$	5-CF_3	111–114	203	$-CH(CH_3)-$	5-Cl	86–88
200	$-CH_2S-$	-	86–88	204	covalent bond	-	not isolated
201	$CH(CH_3)-$	-	117–118	205	*m*-$CH_2C_6H_4$	-	*b*
202	$-CH(CH_3)-$	5-CF_3	105–106	206	*p*-$CH_2C_6H_4$	-	*b*

[a] Structures of compounds confirmed by NMR and MS. [b] Melting points for these and compounds 179–198 were not determined.

ering a range of lipophilicity showed much diminished in vitro activity.

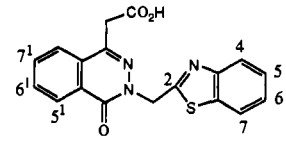
On the basis of this experience we were tempted to shift our attention to the other electrophilic center and prepare 4-substituted compounds. In the meantime, we were highly attracted to draw an analogy from the phenothiazine class of antipsychotic drugs in which the juxtapositional relationship of S and N is the same as in benzothiazole. In both chlorpromazine and triflupromazine, the beneficial substituents are para to the S atom, even though these compounds, as well as the parent phenothiazine (perazine), are metabolized by hydroxylation para to the N atom.[30] This analysis led to the preparation of 5-substituted congeners. Incorporation of Br, the best substituent so far, at the 5-position gave **215** with the first clear-cut jump in potency. In sciatic nerve, it had an ED_{50} of 5.6 mg/kg in the chronic model, suggesting that 5-substituted compounds may show a sufficiently long half-life in man for once-a-day dosing schedule. Replacement of 5-Br by 5-CF_3, spawning a S, N, and CF_3 pattern found in triflupromazine (**9**), led to the discovery of our clinical candidate, zopolrestat (**216**).

Structure–Activity Relationships (SAR)

SAR encompassed substituents on the benzo portion of benzothiazole side chain, spacers between the phthalazinone ring and side chain, substituents on the benzo ring of phthalazinone, and modifications of the acetic acid moiety, including the carboxyl group. Results are discussed both with respect to in vitro and in vivo tests. Biological data for target compounds are shown in Table V.

(30) (a) *Psychotherapeutic Drugs, Part I*: Usdin, E.; Forrest, I. E., Eds.; Marcel Dekker, Inc.: New York, 1976; pp 699–744. (b) Fishman, V.; Heaton, A.; Goldenberg, H. *Proc. Soc. Exp. Biol. Med.* **1962**, *109*, 584.

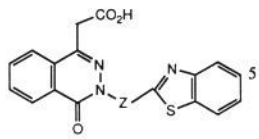
Table V. Physical and Biological Data for Substituted 3,4-Dihydro-4-oxophthalazine-1-acetic Acids



compd	subst	formula	mp, °C	anal.	IC_{50},[a] M	inhibn of sorbitol accumulation in vivo[b]	
						dose, mg/kg	% inhibn
207		$C_{18}H_{13}N_3O_3S$	205 (d)	C, H, N	1.9×10^{-8}	25	67
208	4-F	$C_{18}H_{12}FN_3O_3S$	217–218	C, H, N	2.0×10^{-9}	10	NS[c]
209	4-Cl	$C_{18}H_{12}ClN_3O_3S$	217 (d)	C, H, N	3.5×10^{-9}	10	63
210	4-CF_3	$C_{19}H_{12}F_3N_3O_3S$	198	C, H, N	2.5×10^{-8}	10	61
211	4-OH	$C_{18}H_{13}N_3O_4S$	165–166	C, H, N	1.0×10^{-7}	25	NS
212	4-OMe	$C_{19}H_{15}N_3O_4S$	200–201	C, H, N	1.0×10^{-7}	25	NS
213	5-F	$C_{18}H_{12}FN_3O_3S$	222 (d)	C, H, N	6.9×10^{-9}	5	60
214	5-Cl	$C_{18}H_{12}ClN_3O_3S$	210–212	C, H, N	7.1×10^{-9}	10	73
215	5-Br	$C_{18}H_{12}BrN_3O_3S$	214	C, H, N	3.1×10^{-9}	10	87
216	5-CF_3	$C_{19}H_{12}F_3N_3O_3S$	197–198	C, H, N	3.1×10^{-9}	10	80
217	5-Me	$C_{19}H_{15}N_3O_3S$	205 (d)	C, H, N	2.6×10^{-8}	25	NS
218	5-OH	$C_{18}H_{13}N_3O_4S$	165–166	*d*	4.1×10^{-8}		NT[e]
219	5-OMe	$C_{19}H_{15}N_3O_4S$	195	C, H, N	2.3×10^{-7}	25	NS
220	5-SMe	$C_{19}H_{15}N_3O_3S_2$	187–188	C, H, N	6.0×10^{-8}	10	NS
221	5-SOMe	$C_{19}H_{15}N_3O_4S_2$	184 (d)	C, H, N[f]	4.3×10^{-6}		NT
222	5-SO_2Me	$C_{19}H_{15}N_3O_5S_2$	210–211 (d)	C, H, N	1.7×10^{-6}	10	NS
223	6-Cl	$C_{18}H_{12}ClN_3O_3S$	217 (d)	C, H, N	7.6×10^{-8}	25	61
224	6-Br	$C_{18}H_{12}BrN_3O_3S$	214	C, H, N	2.6×10^{-8}	10	55
225	6-CF_3	$C_{19}H_{12}F_3N_3O_3S$	194–195	C, H, N	7.0×10^{-8}	10	65
226	6-Me	$C_{19}H_{15}N_3O_3S$	202 (d)	C, H, N	3.2×10^{-7}	25	NS
227	6-OH	$C_{18}H_{13}N_3O_4S$	211	C, H, N	1.0×10^{-6}		NT
228	6-OMe	$C_{19}H_{15}N_3O_4S$	189 (d)	*d*	4.2×10^{-6}		NT
229	6-*i*Pr	$C_{21}H_{19}N_3O_3S$	160–161	C, H, N	3.2×10^{-6}		NT
230	7-Cl	$C_{18}H_{12}ClN_3O_3S$	168	C, H, N	1.0×10^{-9}	10	52
231	7-Br	$C_{18}H_{12}BrN_3O_3S$	173–175	C, H, N	1.5×10^{-8}	10	71
232	7-CF_3	$C_{19}H_{15}N_3O_3S$	177–178	C, H, N	1.0×10^{-8}	10	80
233	4,5-F_2	$C_{18}H_{11}F_2N_3O_3S$	178–181	*d*	1.0×10^{-8}	10	82
234	4,5-Cl_2	$C_{18}H_{11}Cl_2N_3O_3S$	222	C, H, N	1.0×10^{-7}	10	49
235	4,5-benzo	$C_{22}H_{17}N_3O_3S$	192–194	*g*	5.1×10^{-7}	25	NS
236	5,6-F_2	$C_{18}H_{11}F_2N_3O_3S$	222	C, H, N	6.8×10^{-8}	25	75
237	5-Cl,6-F	$C_{18}H_{11}ClFN_3O_3S$	207	*d*	1.1×10^{-7}	10	NS
238	5,6-Cl_2	$C_{18}H_{11}Cl_2N_3O_3S$	192–195	*d*	4.2×10^{-7}	25	34
239	5-OMe,6-F	$C_{19}H_{14}FN_3O_4S$	220	C, H, N	2.3×10^{-6}	25	NS
240	5-CF_3,6-OH	$C_{19}H_{12}F_3N_3O_4S$	220 (d)	C, H, N[h]	1.2×10^{-7}	25	NS
241	5-CF_3,6-OMe	$C_{20}H_{14}F_3N_3O_4S$	210–211	C, H, N	4.3×10^{-6}		NT
242	6-Me,7-F	$C_{19}H_{14}FN_3O_3S$	210	C, H, N[i]	3.2×10^{-7}		NT
243	4,6-Cl_2	$C_{18}H_{11}Cl_2N_3O_3S$	192–195	*d*	1.7×10^{-7}		NT
244	4,7-F_2	$C_{18}H_{11}F_2N_3O_3S$	191	*d*	5.0×10^{-9}	10	73
245	4,7-Cl_2	$C_{18}H_{11}Cl_2N_3O_3S$	223–224	C, H, N	2.0×10^{-8}	25	65
246	5,7-F_2	$C_{18}H_{11}F_2N_3O_3S$	178	C, H, N	6.3×10^{-9}	10	80
247	5-F,7-Cl	$C_{18}H_{11}ClFN_3O_3S$	127	C, H, N	6.2×10^{-9}	10	58
248	5,7-Cl_2	$C_{18}H_{11}Cl_2N_3O_3S$	213	C, H, N	4.0×10^{-9}	10	73
249	5-Cl,7-F	$C_{18}H_{11}ClFN_3O_3S$	206	C, H, N	5.0×10^{-9}	10	63
250	5-F,7-CF_3	$C_{19}H_{11}F_4N_3O_3S$	176–177	*d*	1.0×10^{-7}	10	NS
251	5-Cl,7-CF_3	$C_{19}H_{11}ClF_3N_3O_3S$	186–187	C, H, N[j]	6.2×10^{-7}		NT
252	5-CF_3,7-F	$C_{19}H_{11}F_4N_3O_3S$	167–171	*d*	1.7×10^{-8}		NT
253	5-CF_3,7-Cl	$C_{19}H_{11}ClF_3N_3O_3S$	206–208	C, H, N[k]	9.4×10^{-8}	10	26
254	5,7-$(CF_3)_2$	$C_{20}H_{11}F_6N_3O_3S$	186–187	*d*	1.4×10^{-6}	10	NS
255	5,7-Me_2	$C_{20}H_{17}N_3O_3S$	205	C, H, N	5.9×10^{-9}	25	39
256	5-OMe,7-CF_3	$C_{20}H_{14}F_3N_3O_4S$	213 (d)	C, H, N	6.6×10^{-7}		NT
257	5,7-$(OMe)_2$	$C_{20}H_{17}N_3O_5S$	219–220	C, H, N	7.0×10^{-7}		NT
258	5,7-$(OH)_2$	$C_{18}H_{13}O_5N_3S$	184	C, H, N	8.7×10^{-8}		NT
259	7-aza	$C_{17}H_{12}N_4O_3S$	176–177	C, H, N	1.0×10^{-8}	25	83
260	5′-F	$C_{18}H_{12}FN_3O_3S$	204–205	*d*	3.0×10^{-8}		NT
261	6′-Cl	$C_{18}H_{12}ClN_3O_3S$	198–199	*d*	1.5×10^{-8}		NT
262	7′-Cl	$C_{18}H_{12}ClN_3O_3S$	199	C, H, N	3.4×10^{-8}		NT
263	6′-Br	$C_{18}H_{12}BrN_3O_3S$	211	*d*	6.9×10^{-7}	10	42
264	7′-Br	$C_{18}H_{12}BrN_3O_3S$	192	C, H, N	1.2×10^{-8}	10	NS
265	6′-NO_2	$C_{18}H_{12}N_4O_5S$	199–201	*d*	3.3×10^{-8}	10	NS
266	7′-NO_2	$C_{18}H_{12}N_4O_5S$	155–158	*d*	4.6×10^{-8}	10	NS
267	5′-Me	$C_{19}H_{15}N_3O_3S$	201–203	C, H, N	4.2×10^{-7}	10	NS
268	7′-Me	$C_{19}H_{15}N_3O_3S$	187–190	*d*	2.7×10^{-8}		NT
269	6′-OMe	$C_{19}H_{15}N_3O_4S$	177–179	*d*	2.6×10^{-8}	10	NS
270	7′-OMe	$C_{19}H_{15}N_3O_4S$	198–202	*g*	2.8×10^{-8}		NT
271	6′-*i*Pr	$C_{21}H_{19}N_3O_3S$	184–185	C, H, N	1.1×10^{-7}	5	NS
272	7′-*i*Pr	$C_{21}H_{19}N_3O_3S$	99–101	*d*	1.1×10^{-7}		NT

Table V (Continued)

compd	subst	formula	mp, °C	anal.	IC_{50},[a] M	inhibn of sorbitol accumulation in vivo[b] dose, mg/kg	% inhibn
273	6′-CF_3	$C_{19}H_{12}F_3N_3S$	210–211	C, H, N	1.2×10^{-8}	10	NS
274	7′-CF_3	$C_{19}H_{12}F_3N_3S$	124–126	*g*	1.8×10^{-8}	10	NS
275	6′,7′-Cl_2	$C_{18}H_{11}Cl_2N_3O_5S$	189–192	C, H, N	1.0×10^{-8}	10	63
276	4,5-benzo, 7′-Cl	$C_{22}H_{16}FN_3O_3S$	218–222	*g*	3.0×10^{-7}		NT
277	4,5-benzo, 7′-Cl	$C_{22}H_{16}ClN_3O_3S \cdot 1.5H_2O$	209–210	C, H, N	3.5×10^{-7}		NT
278	4,5-benzo, 5′-Me	$C_{23}H_{18}FN_3O_3S \cdot 1.5H_2O$	215–219	C, H, N	4.2×10^{-8}	10	NS
279	6-Br, 6′-,7′-Cl_2	$C_{18}H_{10}BrClN_3O_3S \cdot 1.5H_2O$	206 (d)	C, H, N	1.8×10^{-6}		NT



compd	Z	subst	formula	mp, °C	anal.	IC_{50},[a] M	inhibn of sorbitol accumulation in vivo[b] dose, mg/kg	% inhibn
280	$-CH_2CH_2-$	5-CF_3	$C_{20}H_{14}F_3N_3O_3S$	185	C, H, N	4.6×10^{-7}		NT
281	$-CH_2S-$		$C_{18}H_{13}N_3O_3S_2$	158–160	*d*	8.1×10^{-8}		NT
282	$-CH(CH_3)-$		$C_{19}H_{15}N_3O_3S$	159–160 (d)	C, H, N	4.3×10^{-8}	25	50
283	$-CH(CH_3)-$	5-CF_3	$C_{20}H_{14}F_3N_3O_3S$	182–183	C, H, N	3.7×10^{-8}	10	36
284	$-CH(CH_3)-$	5-Cl	$C_{19}H_{14}ClN_3O_3S$	205	*d*	2.6×10^{-8}	10	53
285	*m*-$CH_2C_6H_4$		$C_{24}H_{17}N_3O_3S$	211–212	*d*	5.7×10^{-6}		NT
286	*p*-$CH_2C_6H_4$		$C_{24}H_{17}N_3O_3S$	215–216	*d*	$>10^{-5}$		NT
287	covalent bond		$C_{17}H_{11}N_3O_3S$	200 (d)	*d*	$>10^{-5}$		NT

[a] IC_{50}s were calculated with a log linear-regression analysis. The standard agents sorbinil (s), ponalrestat (p), and tolrestat (t) showed the following IC_{50} (M) values: (s) $3.47 \times 10^{-7} \pm 0.25 \times 10^{-7}$ ($n = 120$), (p) $1.1 \times 10^{-7} \pm 0.3 \times 10^{-7}$ ($n = 6$), (t) $1.5 \times 10^{-8} \pm 0.2 \times 10^{-8}$ ($n = 8$). Sorbinil was used as a positive control in all determinations. [b] For protocol, see acute in vivo evaluation in the Experimental Section under Biological Methods. Inhibition values for ponalrestat (**3**), in three tests, were 44, 47, and 57% at 5 mg/kg. [c] NS = not significant at $p < 0.05$ (Student's *t* test). [d] High-resolution mass spectra within accepted limits for M^+ or $M^+ - CO_2$. [e] NT = not tested. [f] C: calcd, 55.19; found, 54.72. [g] Homogenous by TLC and structure confirmed by NMR. [h] C: calcd, 52.42; found, 51.81. [i] C: calcd, 59.52; found, 60.21. [j] C: calcd, 50.29; found, 49.11. [k] C: calcd, 50.29; found, 49.87.

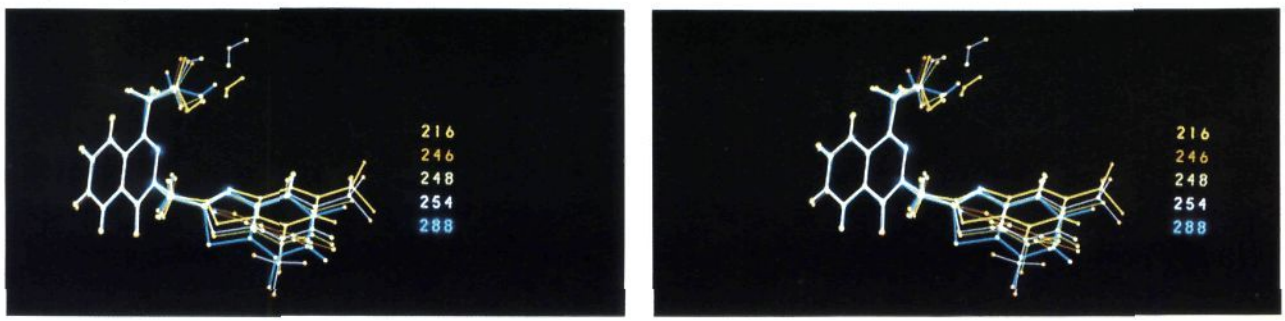
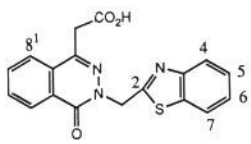


Figure 1. Overlap of X-ray conformations.

Table VI. MMP_2 Steric Energy and Dihedral Angle Data



compd	subst	dihedral angle, deg N–N–C–S	dihedral angle, deg N–N–C–S	steric energy, kcal/mol X-ray	steric energy, kcal/mol 180° rotated
216	5-CF_3	81	51	16.86	17.24
246	5,7-F_2	91	−165	17.68	18.19
248	5,7-Cl_2	89	60	28.72	28.38
254	5,7-$(CF_3)_2$	84	56	13.43	12.98
288	5,7-F_2 (8′-aza)	96	75	*b*	*b*

[a] X-ray data. [b] Not calculated.

In Vitro. Substitution at the 4-carbon of benzothiazole by compact lipophilic groups F, Cl, and CF_3 yielded highly potent compounds (**208**, **209**, and **210**). Polar substituents, (OH, **211**, and OMe, **212**) at that position led to less potent compounds. Similar results prevailed for 5-substituted compounds; Cl and CF_3 analogues (**214** and **216**) were highly potent. In fact, the latter was one of the most potent compounds from this program (vide infra). Compound **217** with a lipophilic but electron-releasing Me group was quite potent. A hydroxyl group at the 5-position (**218**) was better than bulkier or bulkier and polar groups (cf. **219** and **221**). Compounds with 6-substituents were generally less potent than the corresponding 4-, 5-, or 7-substituted compounds. For example, 6-Cl (**223**) was the least potent of the four possible mono-Cl congeners. Again polar or bulky substituents led to less active compounds (**227** and **229**). Substitution at the 7-position was limited to electron-withdrawing groups, Cl, Br, and CF_3; the 7-Cl compound (**230**) was more potent than the bulkier Br and CF_3 analogues.

Among vicinally disubstituted analogues, 4,5-pairs were more potent than the corresponding 5,6-pairs and the pair with greater steric demand was less potent. Thus, **233** was more potent than **236** and **234** was more potent than **238**. Further confirmation of steric effect on activity was observed with the 4,5-benzo compound **235**, which was even

less potent than **234**.

In agreement with SAR among 6-monosubstituted congeners, disubstituted analogues featuring a 6-substituent, especially polar and/or bulky ones, were generally less active. This was so in **240** and **241**.

5,7-Disubstituted compounds with F and Cl were the most potent. In fact, all four possible compounds (**246–249**) were just about as potent as **216**. The dramatic and subtle influence of steric factor on activity was best exemplified by **254**, which was considerably less potent than **255**. The high activity of **255** was consistent with the earlier observed activity of the 5-Me analogue (**217**), but the lower activity of **254** was surprising. While it is usually assumed that CF_3 and Me are sterically interchangeable, as bioisosteres, the van der Waals volume of CF_3 (30,74 Å^3) is almost twice that of Me (15.98 Å^3).[31] Therefore, it appears that the concomitant presence of two CF_3 groups as in **254** places an unfavorable steric demand for interaction with the enzyme. The steric aspect appears to be even more subtle in the 5,7-disubstituted pattern, because incorporation of a 7-CF_3 to potent 5-substituted congeners resulted in less potent compounds (cf. **250** vs **251**) and addition of a 7-substituent onto the 5-CF_3 analogue caused a diminution in potency of that compound (**216** vs **252**). In both instances, the bulkier pair was less potent (**250**/**252** vs **251**/**253**). Addition of 7-CF_3 to 5-substituted analogues caused a greater reduction in biological activity than in the reverse case (**251** vs **253**).

As expected from the above experience, 4,7-difluoro and -dichloro congeners were quite active and the latter was slightly less potent (**244** vs **245**).

SAR pertinent to the spacer included covalent, two carbon, methylenethio, benzene, and branched methylene bridges. Covalent and benzene-bridged modifications showed poor activity. Two carbon, methylenethio, and branched methylene variants retained good activity but were less potent than the corresponding methylene bridged compounds. In the case of branched analogues (e.g. **283**), no attempt was made to prepare optically pure isomers to determine effect of stereochemistry on biological activity.

Substitution on the benzo portion of phthlazinone ring spanned 5′, 6′, and 7′ and included electron-withdrawing/-releasing, polar, and lipophilic substituents. Most showed good activity, but the best members of this subseries were still less potent than the best members of the subseries featuring substituents exclusively on the benzothiazole side chain (e.g. **261** vs **216**). A bulky group such as *i*Pr at either 6′ or 7′ yielded less potent congeners. Steric effect on activity appeared to be more conspicuous for 5′-substitution, although an electronic effect could not be discounted. The vicinally substituted dichloro compound (**275**) was quite potent.

A limited number of both benzothiazole and phthlazinone ring substituted compounds were studied. Results were consistent with SAR observed for the individual parts. However, **276** was less potent than would have been predicted.

Several modifications of the acetic acid moiety were studied. The nor (**48**), vinylogous (**49b**), and homologous (**49c**) acids corresponding to **216** and the branched acid **53** were all far less potent than **216**. Except for **48**, which had an $IC_{50} = 5.0 \times 10^{-6}$ M, the others showed poor inhibition in the range of 1–39% at 10^{-5} M. The dramatic adverse effect of the α-Me group in **53** (cf. **214**) was not surprising in view of a similar experience in the ponalrestat series.[6] Replacement of carboxyl by traditionally used bioisostere tetrazole (**52**) resulted in significant loss of activity.

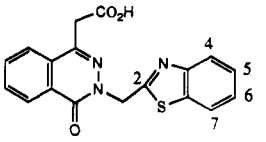
7-Aza analogue **259** was just as potent as the prototype **207**, suggesting that other commonly used bioisosteres (e.g. thiophene) may be compatible with activity.

The somewhat more basic and less planar 2,3-dihydrobenzothiazole congener **46** was a poor inhibitor ($IC_{50} = 5.5 \times 10^{-6}$ M).

In Vivo. The experience in the ARI area to date has been that it is very difficult to obtain good translation of in vitro activity to in vivo activity in models of diabetic complications, particularly among compounds outside of hydantoin family. While a plethora of carboxylic acids have shown high in vitro activity,[4] very few have been reported to show attractive in vivo potency. Our experience in the new series was highly encouraging. Because of paucity of good lens activity among carboxylic acids, only inhibition of sorbitol accumulation in sciatic nerve is given in biological activity tables.

All compounds which showed inhibition of human placental aldose reductase at concentrations lower than 10^{-6} M were tested orally in the rat acute model. Several of the most active compounds were dose titrated to determine ED_{50}s (Table VII). As discussed earlier, **207** exhibited an ED_{50} of 18.5 mg/kg. In the solely benzothiazole-substituted subseries, the more potent (in vitro) and lipophilic compounds were generally more potent than **207** in the acute test. Thus 5-halogen (**213**, **214**), CF_3 (**216**), and 5,7-dihalogen compounds (**246–249**) showed greater than 50% inhibition at 10 mg/kg. However, 5-Me and 5,7-Me_2 analogues (**217** and **255**) were significantly less potent, presumably because of greater susceptibility to metabolism. Methoxy and hydroxy compounds were not active

Table VII. Sciatic Nerve ED_{50}s of Selected (Benzothiazolylmethyl)phthalazinoneacetic Acids in the Acute and Chronic Tests



compd	subst	sciatic nerve ED_{50}, mg/kg: acute[a]	chronic[b]
207	–	18.5	*c*
213	5-F	5.3	5.3
215	5-Br	5.6	4.9
216	5-CF_3	3.6	2[d]
246	5,7-F_2	3.2	4[d]
248	5,7-Cl_2	3.5	2.8
sorbinil (1)		*e*	0.8[d]
tolrestat (2)		*c*	26[d]

[a] Male Sprague–Dawley rats (n = 4) were treated with streptozotocin (85 mg/kg iv) and orally dosed with aqueous suspensions or solutions of aldose reductase inhibitors at 4, 7, and 24 h. Animals were sacrificed at 27 h and the mean sorbitol content of the sciatic nerve and the lens was determined enzymatically and expressed as a percent inhibition of that in untreated diabetic rats. ED_{50}s were calculated by using a log linear regression analysis. [b] Male Sprague–Dawley rats (n = 5) were treated with streptozocin (85 mg/kg iv) and left untreated for 7 days. Aqueous suspensions of solutions of the aldose reductase inhibitors were orally administered s.i.d. for 5 days. Three hours following the last dose, rats were sacrificed, and the sorbitol content of the sciatic nerve was determined by using an enzymatic assay. Sorbitol content was expressed as a percentage of that in untreated diabetic rats. [c] Not determined. [d] Values are the means of three separate experiments with five animals per treatment group. [e] For sorbinil in this test, percent inhibition of sorbitol accumulation was 40.1 ± 2.3% at 0.25 mg/kg (n = 11) and 71.2 ± 10.7% at 0.75 mg/kg (n = 8).

(31) Motoc, I.; Marshall, G. R. *Chem. Phys. Lett.* **1985**, *116*, 415.

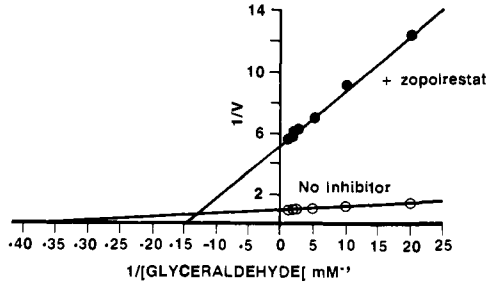


Figure 2. Inhibition of human placenta aldose reductase with zopolrestat. Reaction mixtures contained 50 mM potassium phosphate, pH 7.1, 0.4 M ammonium sulfate, 0.067 mM NADPH, and 0.5 mM DL-glyceraldehyde. NADPH oxidation was monitored spectrophotometrically for 10 min at 25 °C.

at the doses tested. In these cases, it is surmised that problems associated with oral absorption, metabolism, and penetration into target tissues were contributing factors. Poor oral activity of phenolic compounds has been attributed to erratic absorption and poor half-life.[4] The bridge variants **282–284** were reasonably potent given the possibility that activity could reside exclusively in one optical isomer.

Among members of the other subseries tested (phthalazinone substitution only), oral activity was seen only with **263** and **275**. However, the highest dose used was 10 mg/kg. While the observations with the NO_2 (**165** and **266**) and Me (**267** and **268**) analogues could be attributed to high polarity and/or metabolism, the results with **273** and **274**, were surprising when contrasted with those of **216**. This could be attributed to regional lipophilicity which may play an important role in translation of in vitro to in vivo activity.

7-Aza analogue **259**, a more polar analogue of **207**, was at least as potent as **207**.

Selected candidates with low ED_{50}s in the acute test were dose titrated in the more demanding chronic test and ED_{50}s were determined. As can be seen from a comparison of data in Table VII, the compounds showed nearly identical ED_{50}s in the two tests, demonstrating that the compounds were equally effective in either preventing or reversing the accumulation of sorbitol under diabetic conditions.

Zopolrestat

Further in vitro and in vivo pharmacology and pharmacokinetics in rats were undertaken with the title compound which permitted its progression to kinetic studies in man. Results of pharmacology studies and a summary of rat and human pharmacokinetic data are presented below.

A comparison of the initial rates of reduction of glyceraldehyde by human placental aldose reductase in the presence and absence of zopolrestat indicates that the compound is a reversible, noncompetitive inhibitor relative to glyceraldehyde with a K_i of 1.9×10^{-8} M (Figure 2). The inhibition pattern is similar to those reported for a variety of structurally distinct aldose reductase inhibitors.[32] The inhibition of human placenta and rat lens aldose reductase by varying concentrations of zopolrestat at fixed

(32) (a) Okuda, J.; Miwa, I.; Inakagi, K.; Horie, T.; Nakayama, M. *Biochem. Pharmacol.* **1982**, *31*, 3807. (b) Jedziniak, J. A.; Kinoshita, J. H. *Invest. Opthalmol.* **1971**, *10*, 357. (c) Varma, S. D.; Kinoshita, J. H. *Biochem. Pharmacol.* **1976**, *25*, 2505. (d) Kador, P. F.; Sharpless, N. E. *Biophys. Chem.* **1978**, *8*, 81. (e) Inagaki, K.; Miwa, I.; Yashiro, T.; Okuda, J. *Chem. Pharm. Bull.* **1982**, *30*, 3244. (f) Okuda, J.; Miwa, I.; Inagaki, K.; Horie, T.; Nakayama, M. *Chem. Pharm. Bull.* **1984**, *32*, 767.

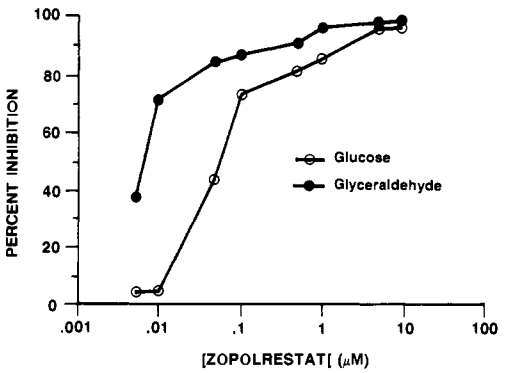


Figure 3. Inhibition of human placenta aldose reductase by zopolrestat. Reaction conditions are described in Figure 2 except that 1.0 M glucose was used as the substrate in the appropriate incubations.

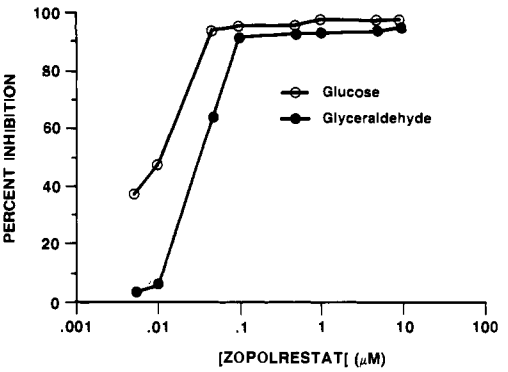


Figure 4. Inhibition of rat lens aldose reductase by zopolrestat. Rat lenses were homogenized in 0.1 M potassium phosphate buffer, pH 7.1, containing 5 mM mercaptoethanol (0.4 mL/lens) and centrifuged at 40000*g* for 3 min. The supernatant was used as the crude aldose reductase. Reaction conditions are as described in Figure 2.

Table VIII. Inhibition of Human Placenta and Rat Lens Aldose Reductase with Zopolrestat

enzyme source	subtrate	IC_{50}, M
human placenta	glyceraldehyde	1.9×10^{-9}
	glucose	8.1×10^{-8}
rat lens	glyceraldehyde	4.1×10^{-8}
	glucose	1.0×10^{-8}

concentrations of substrates (glucose and glyceraldehyde) and NADPH are shown in Figures 3 and 4. The IC_{50} values calculated from this data (Table VIII) show that zopolrestat is a potent inhibitor of the reduction of both glyceraldehyde and glucose by the human and rat enzymes.

When isolated erythrocytes are incubated in high-glucose medium, the cells will accumulate sorbitol in a time-dependent manner. The ability of zopolrestat to inhibit sorbitol accumulation was examined in washed human and rat erythrocytes. Results of these studies and the experimental protocol are summarized in Figure 5. The IC_{50}s for inhibition of sorbitol accumulation were calculated as 3.5×10^{-7} and 2.2×10^{-7} M, respectively.

The ability of zopolrestat to reverse the accumulation of sorbitol, in nerve, lens, and retina, upon once-a-day oral administration, over an expanded dose range of 2.5–50 mg/kg, was studied in the chronic test. Results are shown in Figure 6. The inhibitor showed no effect on blood glucose but caused a dose-dependent decrease in nerve, retina, and lens sorbitol levels. Typical of carboxylic acid ARIs, our compounds, including **216**, were less potent in lens than in nerve. As can be seen from Figure 6, zopolrestat showed nearly a 10-fold difference in ED_{50} between

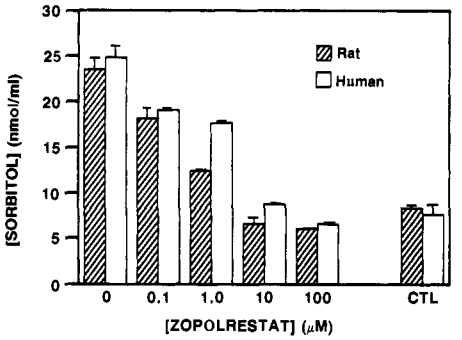


Figure 5. Inhibition of sorbitol accumulation in cultured erythrocytes by zopolrestat. Human blood from fed, male volunteers and rat blood from 200–300-g, fed Sprague–Dawley rats was collected in 10-mL vacutubes containing 14 mg EDTA. Red blood cells were collected by centrifugation and washed three times in 0.9% saline. Two milliliters of packed cells were added to each flask with 4 mL of Krebs–Hensleit bicarbonate buffer, pH 7.4, containing 4% BSA, 50 mM glucose and the indicated concentration of zopolrestat. Control incubations at physiological glucose concentration (5.5. mM) were identical except that no drug was included. Cells were incubated for 2 h at 37 °C with constant gassing with 95:5 oxygen carbon dioxide and were then collected by centrifugation and washed twice with saline. The sorbitol content of a perchloric acid extract of each sample was determined enzymatically. Data are means of four determinations expressed as nmol of sorbitol/mL of packed red cells ± standard deviation.

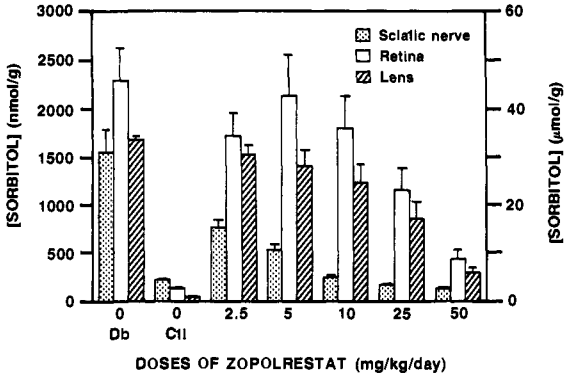


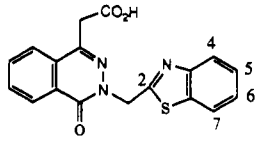
Figure 6. Dose response of zopolrestat in the chronic test. Rats were made diabetic by iv injection of 85 mg/kg streptozotocin. After 7 days, zopolrestat was administered once-a-day by oral gavage to groups of five rats at the indicated doses. Animals were sacrificed 3 hours after dosing on day 12. The sorbitol content of perchloric acid extracts of the sciatic nerve, retina, and lens was determined enzymatically. Data are presented as means ± SEM. ED_{50} values were calculated using a log linear regression analysis; they were 1.9, 17.6, and 18.4 mg/kg for sciatic nerve, retina, and lens, respectively.

nerve and lens (1.9 vs 18.4 mg/kg). This differential in potency is not surprising because the lens is not directly perfused by blood, rather it bathes in aqueous humor. The blood supply is that to the ciliary body. Orally administered drugs upon entering systemic circulation have to cross the blood–aqueous humor barrier and then reach the lens. Carboxylic acid drugs have low pK_a's and are largely in the ionized state in blood (pH ~7.4) and the more lipophilic ones are highly protein bound. These factors work in concert to deter drug transport across the blood–lens barrier.

Turning to our acids, the pK_a's and partition coefficients of **216** and some of its congeners are listed in Table IX.[33]

(33) (a) log *D* values describe the partitioning of the compound between octanol and a buffer at pH 7.4 (b) Smith, D. A.; Brown, K.; Neale, M. G> *Drug. Metab. Rev.* **1985–86**, *16*, 365.

Table IX. pK_a and Lipophilicity Data for Selected Compounds



compd	subst	pK_a (dioxane/water) 1:1	pK_a (dioxane/water) 2:1	log P^a	log D^b
12a		5.49	6.35	2.42	NM[c]
213	5-F	5.49	6.35	2.95	−0.30
215	5-Br	NM	6.35	3.35	NM
216	5-CF_3	5.46	6.38	3.43	0.55
246	5,7-F_2	5.47	6.38	3.01	0.05

[a] *n*-Octanol/water. [b] *n*-Octanol/pH 7.4 buffer. [c] NM not measured.

Unsubstituted acid **12a**, which was sufficiently soluble in water, showed a pK_a of 3.73. Therefore, we believe that **216** and its congeners would be about 5–6 times more acidic than phenylacetic acid in water. **216** was also quite lipophilic and was 97.3% bound to rat plasma proteins at a concentration of 50 μg/mL.[34] Drug levels measured in the nerve and lens from streptozotocin-treated rats, with representative compounds (data not shown), showed roughly a 10-fold higher concentration in nerve than in lens. Following five (50 mg/kg) consecutive once-daily oral administrations of zopolrestat, the peak concentrations in the sciatic nerve and lens were 8370 and 843 ng/g, respectively.[33]

The higher ED_{50} for retina (17.6 mg/kg) is also consistent with the expected poor transport of zopolrestat across the blood–retinal barrier. However, the drug concentration in the retina was not measured.

Zopolrestat was also found to prevent accumulation of sorbitol in the kidney cortex of diabetic rats and normalize elevated renal blood flow in galactosemic rats.[35,36]

Zopolrestat was well absorbed both in the rat and in diabetic patients. In diabetic patients following 10 consecutive daily oral doses of 1000 mg, significant blood level was observed even at 24 h post last dosing. The plasma half-life was 27.5 h.[34] The highly favorable pharmacokinetic results from the human study bode well for ongoing clinical evaluation of zopolrestat in diabetic complications, with once-a-day treatment schedule.

Conclusion

We have discovered that benzothiazole is a new, effective pharmacophore for the design of potent aldose reductase inhibitors. This discovery coupled with medicinal chemical rationale and analogy to part structures of known drugs resulted in the design of zopolrestat, which was highly effective in animal models of diabetic complications. This compound showed extremely good oral absorption, high blood level, and favorably long plasma half-life in diabetic patients. On the basis of these data and preclinical safety, zopolrestat is now being evaluated in the clinic for efficacy in diabetic complications.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point

(34) We thank Dr. P. B. Inskeep of our Metabolism Department for these data.
(35) Beyer, T. A.; Siegel, T. W.; Beebe, D. A.; Aldinger, C. E.; Ellery, C. A.; Ashton, M. A.; Pustilnick, L. R.; Morehouse, L. A. *Diabetes* **1990**, *39(suppl. 1)*, 187A.
(36) Oates, P. J.; Ellery, C. A. *Diabetes* **1990**, *39(suppl. 1)*, 184A.

apparatus and are uncorrected. Structures of all new compounds were confirmed by NMR and MS spectra. ^{1}H NMR spectra were obtained on Bruker (AM300) or Varian (XL250 or T69) instruments. Chemical shifts are expressed in ppm downfield from internal TMS. ^{1}H NMR data are tabulated in the following order: chemical shift, multiplicity, number of protons, coupling constant(s) in hertz. High resolution mass spectra were run on a Kratos (MS30) high-resolution mass spectrometer. Satisfactory elemental analysis was obtained on all target carboxylic acids except as noted. X-ray data are available as supplementary material.

(*E*)-3-[(Ethoxycarbonyl)methylidene]-5,6-dichlorophthalide (11, 5′,6′-Cl_2). A solution of 4,5-dichlorophthalic anhydride (10.0 g, 46 mmol) and (carbethoxymethylene)triphenylphosphorane (16.0 g, 46 mmol) in $CHCl_3$ (450 mL) was refluxed for 16 h. Evaporation of $CHCl_3$ and purification of the residue by chromatography gave the product (54%): ^{1}H NMR (300 MHz, DMSO) δ 1.1 (t, 3 H, 9), 4.1 (q, 2 H, 9), 6.1 (s, 1 H), 8.2 (s, 1 H), 8.4 (s, 1 H).

Ethyl 6,7-Dichloro-3,4-dihydro-4-oxophthalazine-1-acetate. A mixture of 3-[(ethoxycarbonyl)methylidene]-5,6-dichlorophthalide (9.37 g, 33 mmol), EtOH (300 mL), and hydrazine (1.1 g, 35 mmol) was refluxed for 3 h. Upon cooling, the precipitated solid was collected (89%): mp 250 °C; ^{1}H NMR (300 MHz, DMSO) δ 1.0 (t, 3 H, 9), 4.1 (q, 2 H, 9), 4.0 (s, 2 H), 8.3 (s, 1 H), 8.4 (s, 1 H).

2,5-Dibromothioacetanilide (Cf. 17). A mixture of 2,5-dibromoacetanilide (45.0 g, 0.16 mol), phosphorus pentasulfide (24.4 g, 0.19 mol), and benzene (500 mL) was refluxed for 18 h. After cooling of the reaction mixture, the benzene layer was decanted and then extracted with 10% aqueous KOH (2 × 50 mL), and the aqueous extract was acidified to pH 4.0 by the addition of dilute HCl. The precipitated product was collected and then air-dried (29%): mp 119–124 °C.

2-Methyl-5-bromobenzothiazole (16, 5-Br). To a solution of 2,5-dibromothioacetanilide (12.4 g, 0.04 mol) in *N*-methylpyrrolidinone was cautiously added NaH (1.93 g, 0.04 mol, 50% w/w dispersion in mineral oil). After the solution was complete, the mixture was heated at 150 °C for 1.5 h. The dark solution was poured onto ice/water (300 mL) and the separated brown gum was extracted with EtOAc (2 × 100 mL). The extract was washed with H_2O, dried over anhydrous $MgSO_4$, and then evaporated. The resulting crude solid was purified by chromatography (49%): mp 84–85 °C (lit.[13] mp 87 °C).

2-(Bromomethyl)-5-bromobenzothiazole (77). A mixture of 2-methyl-5-bromobenzothiazole (32.0 g, 0.11 mol), *N*-bromosuccinimide (25.1 g, 0.14 mol), CCl_4 (700 mL), and a catalytic amount of benzoyl peroxide (1.02 g) was refluxed under irradiation by an UV lamp for 14 h. The reaction was cooled and filtered to remove the precipitated succinimide and the filtrate was evaporated to dryness. The resulting solid was purified by chromatography (23%): mp 106–108 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 4.8 (s, 1 H), 7.5 (m, 2 H), 8.1 (d, 1 H, 2).

Ethyl 3,4-Dihydro-4-oxo-3-[(5-bromo-2-benzothiazolyl)methyl]-1-phthalazineacetate (139). Method 1. To a mixture of 12 (11.6 g, 0.05 mol) and NaH (2.38 g, 0.05 mol) in DMF was added 5-bromo-2-(bromomethyl)benzothiazole (16.8 g, 0.055 mol) and the resulting mixture was stirred at room temperature for 1 h. It was then poured over ice/water (500 mL); sufficient 10% HCl was added to adjust the pH to about 4.0 and the precipitated crude solid was dried and crystallized from ethanol to obtain **139** (73%): mp 159 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.3 (t, 3 H, 8), 4.0 (s, 2 H), 4.2 (q, 2 H, 8), 5.8 (s, 2 H), 7.4–8.2 (m, 6 H), 8.4 (m, 1 H).

3,4-Dihydro-4-oxo-3-[(5-bromo-2-benzothiazolyl)methyl]-1-phthalazineacetic Acid (215). A mixture of **139** (15.0 g, 0.035 mol) and dioxane (150 mL) was brought to solution by warming on a steam bath and to this solution was added aqueous 10% KOH (20 mL) in EtOH (50 mL). The resulting purple solution was stirred at room temperature for 2 h and then concentrated to remove excess dioxane and EtOH. The concentrate was diluted with H_2O (100 mL) and the solution was washed with ether (2 × 100 mL). The aqueous layer was collected and acidified to pH 2.0 by addition of concentrated HCl. The precipitated solid was dried and crystallized from CH_2Cl_2/EtOH (400 mL/40 mL) to obtain the product (87%): mp 214 °C; ^{1}H NMR (DMSO, 250 MHz) δ 4.2 (s, 2 H), 5.8 (s, 2 H), 7.65 (dd, 1 H, 2 and 8), 7.9–8.1 (m, 4 H), 8.28 (d, 1 H, 2), 8.4 (dd, 1 H, 2 and 8).

Ethyl 3,4-Dihydro-4-oxo-3-(3-cyanomethyl)-1-phthalazineacetate (36a). To a solution of **12a** (11.31 g, 50 mmol) and dry potassium *tert*-butoxide (5.9 g, 52 mmol) in DMF (50 mL) was added chloroacetonitrile (3.78 g, 52 mmol) and the solution was stirred for 30 min at room temperature. This solution was poured onto ice/water (300 mL); sufficient 10% HCl was added to adjust the pH to about 4.0 and the precipitated solid was collected and crystallized from ethanol (91%): mp 113–114 °C; ^{1}H NMR (DMSO, 300 MHz) δ 1.3 (t, 3 H, 8 Hz), 4.1 (s, 2 H), 4.2 (q, 2 H, 8), 5.4 (s, 2 H), 8.0 (m, 3 H), 8.3 (dd, 1 H, 2 and 8). Compound **36b** (as methyl ester) was prepared in a similar manner, but with chloropropionitrile (74%): mp 125–126 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 3.0 (t, 2 H, 6), 3.7 (s, 3 H), 4.0 (s, 2 H), 4.5 (t, 2 H, 6), 7.8 (m, 3 H), 8.4 (m, 1 H).

Alternative Method for 36a. A mixture of **12** (23.42 g, 0.1 mol), EtOH (200 mL), and aqueous formaldehyde (37%, 100 mL) was refluxed for 40 h. This solution was concentrated to 100 mL and was then poured onto ice/water (750 mL). The precipitated solid, ethyl 3,4-dihydro-4-oxo-3-(hydroxymethyl)-1-phthalazineacetate (**38a**), was collected and air-dried (65%): mp 113–114 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.2 (t, 3 H, 8), 3.9 (s, 2 H), 4.2 (q, 2 H, 2 and 8), 5.6 (s, 2 H), 7.9 (m, 3 H), 8.4 (m, 1 H). A mixture of this solid (13.1 g, 0.05 mol), phosphorus tribromide (13.5 g, 0.05 mol), and anhydrous Et_2O (250 mL) was stirred overnight at room temperature. It was then poured onto H_2O (200 mL). The organic layer was collected, washed again with H_2O (3 × 100 mL), and then dried over anhydrous sodium sulfate. The dried organic extract was evaporated to dryness and the resulting crude solid was purified by chromatography on silica gel, eluting with 95% CH_2Cl_2/5% EtOAC to obtain **39**, ethyl 3,4-dihydro-4-oxo-3-(bromomethyl)-1-phthalazineacetate: (96%): mp 96 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.3 (t, 3 H), 4.0 (s, 2 H), 4.3 (q, 2 H, 8), 6.0 (s, 2 H), 7.8 (m, 3 H), 8.4 (m, 1 H). To an ice-cold solution of **39** (2.43 g, 7.5 mmol) in acetone (3.5 mL) was added dropwise a solution of KCN (0.49 g, 7.5 mmol) and KI (2 mg) in H_2O (3.5 mL). The reaction mixture was stirred for 2 h and it was then poured onto ice/water (200 mL). The precipitated solid was purified by chromatography to obtain the title compound (63%).

Ethyl 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetate (145). Method 2. A mixture of **36a** (2.71 g, 10 mmol), 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride[19] (2.4 g, 10.5 mmol), and EtOH (20 mL) was refluxed for 8 h. The heavy precipitate obtained upon cooling was filtered and the collected solid was air-dried to obtain the product (94%): mp 124–126; ^{1}H NMR (DMSO, 300 MHz) δ 1.2 (t, 3 H, 8), 4.15 (s, 2 H), 4.1 (q, 2 H, 8), 5.8 (s, 2 H), 7.75 (dd, 1 H, 2 and 8), 7.9–8.1 (m, 3 H), 8.9 (m, 3 H).

Preparation of 145. Method 3. To a solution of **6a** (10.84 g, 38 mmol) and a catalytic amount of triethylamine (0.2 g) in DMF (40 mL) maintained at 50–55 °C was bubbled with H_2S for 15 min. After the reaction was continued for 3 h, the solution was resaturated with H_2S and commercially available 4-chloro-3-nitrobenzotrifluoride (9.74 g, 44 mmol) was added. The reaction immediately turned light orange and it was then heated to 140 °C for 2.5 h. The solution was cooled and was added dropwise to a mixture of ice/water and EtOH (800 mL, 4:1). The pH of the mixture was adjusted to about 2.0 with a few drops of 6 N HCl. The resulting granulated solid was crystallized from a 3:1 mixture (50 mL) of EtOH/CH_2Cl_2 (75%).

Ethyl 3-[2-[[2-Bromo-3,5-bis(trifluoromethyl)phenyl]amino]-2-oxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetate (42). Ester **12** (4.4 g, 18.9 mmol), was alkylated according to the procedure for **36a**, using 2′-bromo-3′,5′-bis(trifluoromethyl)-2-chloroacetanilide (**41**: mp 67–68 °C; 7.3 g, 18.9 mmol) in place of chloroacetonitrile. The desired product **42** was obtained as a white, crystalline solid (38%): mp 195–197 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.4 (t, 3 H, 8), 4.1 (s, 2 H), 4.2 (q, 2 H, 8), 5.2 (s, 2 H), 7.6–8.0 (m, 4 H), 8.4 (m, 1 H), 8.6 (m, 2 H).

Ethyl 3-[2-[[2-Bromo-3,5-bis(trifluoromethyl)phenyl]amino]-2-thioxoethyl]-3,4-dihydro-4-oxo-1-phthalazineacetate (43). A mixture of **42** (1.0 g, 1.7 mmol), benzene (10 mL), and phosphorus pentasulfide (0.5 g, 2.4 mmol) was heated at 70 °C for 4 h. The mixture was cooled and filtered, and the filtrate was evaporated to obtain the product (59%): mp 121–122 °C; ^{1}H

NMR ($CDCl_3$, 60 MHz) δ 1.4 (t, 3 H, 8), 4.05 (s, 2 H), 4.1 (q, 2 H), 5.6 (s, 2 H), 7.6–8.1 (m, 4 H), 8.4 (m, 1 H), 8.6 (m, 2 H).

Ethyl 3,4-Dihydro-4-oxo-3-[[3,5-bis(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetate (170). Method 4. To a solution of **43** (0.62 g, 1.0 mmol) in *N*-methylpyrrolidone (5 mL) was added sodium hydride (48 mg, 1 mmol) and then the mixture was heated to 90 °C for 2 h. After cooling, it was diluted with H_2O and extracted with Et_2O. The organic layer was further washed with H_2O several times and evaporated to dryness, and the resulting solid was crystallized from EtOH (62%): mp 186–187 °C; MS *m/e* 515.0709 (M^+). Structure of this product was also confirmed by single-crystal X-ray analysis.

3,4-Dihydro-4-oxo-3-[(2,3-dihydro-2-benzothiazolyl)-methyl]-1-phthalazineacetic Acid (46). Alkylation of **12** (10.91 g, 0.05 mol) with bromoacetaldehyde diethyl acetal (10.83 g, 0.05 mol) as described for **36a** gave 44 (40%); mp 68 °C. It was hydrolyzed with aqueous NaOH to obtain **45** (83%); mp 114–115 °C. A mixture of **45** (0.32 g, 1 mmol), EtOH (5 mL), and 2-aminobenzenethiol hydrochloride (0.23 g, 1 mmol) was refluxed for 6 h and then evaporated to dryness. The residue was extracted with EtOAc and washed with water and the EtOAc layer was dried and evaporated. The resulting solid was crystallized from MeOH to obtain a white solid (53%): mp 134–135 °C.

3,4-Dihydro-4-oxo-3-[(5,7-dihydroxy-2-benzothiazolyl)-methyl]-1-phthalazineacetic Acid (258). A mixture of ethyl 3,4-dihydro-4-oxo-3-[(5,7-dimethoxy-2-benzothiazolylmethyl]-1-phthalazineacetate (171; 0.5 g, 1.2 mmol) and aqueous hydrobromic acid (48%, 10 mL) was refluxed for 4.5 h. Upon cooling, the pink solution was poured onto ice/water and the precipitated solid was collected and crystallized from MeOH (67%): mp 184 °C; ^1H NMR (DMSO, 300 MHz) δ 4.05 (s, 2 H), 5.7 (s, 2 H), 6.4 (s, 1 H), 6.8 (s, 1 H), 8.0 (m, 3 H), 8.4 (d, 1 H, 8), 9.6 (s, 1 H).

Methyl 5-Fluoro-7-(trifluoromethyl)benzothiazole-2-carboxylate (26, R = Me). A solution of methyl 5-amino-7-(trifluoromethyl)benzothiazole-2-carboxylate[16] (2.0 g, 7.25 mmol) in concentrated HCl (10 mL) was cooled to 0 °C in an ice bath and sodium nitrite (0.52 g, 7.5 mmol) was added in small portions. The temperature to the solution was raised to 10 °C and kept at that temperature for 30 min. To this was added hexafluorophosphoric acid (20 mL) and the mixture was stirred vigorously. The precipitated solid was collected and air-dried. This crude diazonium salt was sublimed at 250 °C and the sublimate proudct was collected (31%): ^1H NMR ($CDCl_3$, 250 MHz) δ 4.05 (s, 3 H), 7.7 (dd, 1 H, 2 and 7), 8.0 (dd, 1 H, 2 and 7).

5-Fluoro-7-(trifluoromethyl)-2-benzothiazolylmethanol (Cf. 23c). To a slurry of $LiAlH-(t\text{-}BuO)_3$ (1.3 g, 4.8 mmol) in THF (10 mL) was added methyl 5-fluoro-7-(trifluoromethyl)benzothiazole-2-carboxylate (0.67 g, 2.39 mmol) dissolved in THF (5 mL) and the mixture was allowed to stir for 1 h. The reaction mixture was cautiously quenched with H_2O, the pH was adjusted to around 7, and then the mixture was extracted with EtOAc. The organic layer was washed with H_2O, dried, and then evaporated to dryness to obtain the product (91%), ^1H NMR ($CDCl_3$, 250 MHz) δ 4.1 (s, 2 H), 7.65 (dd, 1 H, 2 and 7).

2-(Chloromethyl)-5-fluoro-7-(trifluoromethyl)benzothiazole (95). To a solution of 5-fluoro-7-(trifluoromethyl)-2-benzothiazolylmethanol (0.55 g, 2.19 mmol) in CH_2Cl_2 (10 mL) containing pyridine (2 mL) was added methanesulfonyl chloride (0.35 mL, 4.38 mmol) and the mixture was stirred overnight at 0 °C. The reaction was quenched with H_2O (20 mL) containing a few drops of concentrated HCl and then extracted with Et_2O (2 × 25 mL). The organic layer was evaporated to obtain the product (65%): ^1H NMR ($CDCl_3$, 60 MHz) δ 4.9 (s, 2 H), 7.4 (dd, 1 H), 7.8 (dd, 1 H, 2 and 8).

6-(Trifluoromethyl)benzothiazole (Cf. 31). To a solution of 2-amino-6-(trifluoromethyl)benzothiazole (6.54 g, 30 mmol) in phosphoric acid (85%, 100 mL) cooled to 0 °C was slowly added a solution of sodium nitride (10.35 g, 150 mmol) in H_2O (20 mL). After stirring of the thick syrupy solution for 30 min, it was gradually added to ice-cold hypophosphorous acid (50%, 100 mL) and then slowly warmed to room temperature. The pH of the reaction mixture was raised to about 6 by addition of solid Na_2CO_3 and the resulting gummy solid was extracted with CH_2Cl_2. The organic layer was washed with H_2O and then evaporated to dryness. The crude solid was purified by chromatography (74%): mp 42 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.8 (dd, 1 H, 2 and 8), 8.2 (s, 1 H), 8.3 (d, 1 H, 8), 9.15 (s, 1 H).

4-(Trifluoromethyl)benzothiazole was prepared starting from 2-amino-4-(trifluoromethyl)benzothiazole (74%), mp 42 °C.

4-Amino-5-mercaptobenzotrifluoride Hydrochloride (115). Hydrazine hydrate (5 mL) was slowly added to a solution of 6-(trifluoromethyl)benzothiazole (2.03 g, 10 mmol) and stirred overnight at room temperature. The solution was concentrated and gradually added to H_2O (50 mL) and the pH of the solution was adjusted to about 2 by the addition of sufficient concentrated HCl. The precipitated yellow solid was collected (89%), mp 210–213 °C.

Ethyl 3,4-Dihydro-4-oxo-3-[3-(2-benzothiazolyl)benzyl]-1-phthalazineacetate (205). A mixture of ethyl 3,4-dihydro-4-oxo-3-(3-cyanobenzyl)-1-phthalazineacetate (0.5 g, 1.4 mmol); mp 141–142 °C; prepared by alkylation of **12a** with 3-cyanobenzyl bromide), 2-aminobenzenethiol hydrochloride (0.23 g, 1.44 mmol), and EtOH (6 mL) was refluxed overnight. The mixture was evaporated to dryness and the residue was purified by chromatography (to obtain 19% of **205**): ^1H NMR (DMSO, 200 MHz) δ 1.1 (t, 3 H, 9), 3.85 (s, 2 H), 4.1 (q, 2 H, 9), 5.3 (s, 2 H), 7.4–7.9 (m, 11 H), 8.4 (d, 1 H, 7).

1-(2-Benzothiazolyl)ethyl Chloride (101). To a solution of 1-(2-benzothiazolyl)ethanol[18] (2.5 g, 9.1 mmol) in methylene chloride (50 mL) was added thionyl chloride (3.32 g, 28 mmol) and the resulting solution was stirred at room temperature for 1 h. The solution was poured onto ice/water (100 mL) and extracted with CH_2Cl_2, and the organic extract separated. This extract was washed with $NaHCO_3$ (10 mL, 5% solution) and then with H_2O (50 mL). The CH_2Cl_2 layer was dried over anhydrous $MgSO_4$ and evaporated to a light yellow oil (2.08 g): ^1H NMR ($CDCl_3$, 60 MHz) δ 1.9 (d, 3 H, 7), 5.4 (q, 1 H, 7), 7.6–7.2 (m, 2 H), 7.9–8.2 (m, 2 H).

1-[5-(Trifluoromethyl)-2-benzothiazolyl]ethanol (Cf. 21a). A mixture of 5-(trifluoromethyl)-2-aminobenzenethiol hydrochloride (5.73 g, 25 mmol) and *d,l*-lactic acid (3.15 g, 35 mmol) was heated at 150 °C under a blanket of N_2. The melt was quenched with ice/water (20 mL) and then basified to pH ~9.0 with aqueous NH_4OH, and the resulting solid was collected (78%): mp 94–96 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 1.9 (d, 3 H, 7), 5.3 (q, 1 H, 7), 7.8 (dd, 1 H, 2 and 8), 8.3 (m, 2 H).

1-[5-(Trifluoromethyl)-2-benzothiazolyl]ethanol Mesylate (103). To an ice-cold solution of 1-[5-trifluoromethyl)-2-benzothiazolyl]ethanol (4.94 g, 20 mmol) in dry pyridine was added methanesulfonyl chloride (4.58 g, 40 mmol) and the resulting solution was stirred at 0 °C for 1 h. It was poured onto H_2O and extracted with Et_2O, and the Et_2O layer was washed with 10% HCl (2 × 20 mL). The organic extract was dried and evaporated and the residue was triturated with hexane to obtain the desired compound (99%): mp 89 °C; ^1H NMR ($CDCl_3$, 60 MHz), δ 1.8 (d, 3 H, 8), 3.0 (s, 3 H), 6.0 (q, 1 H, 7), 7.6 (dd, 1 H, 2 and 10), 7.9 (dd, 1 H, 2 and 7), 8.25 (d, 1 H, 2).

Ethyl 3,4-Dihydro-4-oxo-[[5-(methylsulfinyl)-2-benzothiazolyl]methyl]-1-phthalazineacetate (152). To an ice-cold solution of ethyl 3,4-dihydro-3-[[5-(methylthio)benzothiazol-2-yl]methyll[-4-oxo-1-phthalazineacetate (1.06 g, 2.5 mmol) in $CHCl_3$ (10 mL) was added *m*-chloroperoxybenzoic acid (0.50 g, 2.5 mmol). The resulting solution was stirred at between 0 and 5 °C for 1 h. The $CHCl_3$ solution was washed with 10% $NaHCO_3$ solution (3 × 20 mL); the separated organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography over silica gel to obtain the title compound (73%): ^1H NMR ($CDCl_3$, 60 MHz): δ 1.2 (t, 3 H, 8), 2.65 (s, 3 H), 3.95 (s, 2 H), 4.1 (q, 2 H, 8), 5.75 (s, 2 H), 7.4–8.3 (m, 7 H).

Ethyl 3,4-Dihydro-4-oxo-3-[[5-(methylsulfonyl)-2-benzothiazolyl]methyl]-1-phthalazineacetate (153). A solution of 151 (1.06 g, 2.5 mmol) and *m*-chloroperoxybenzoic acid (1.3 g, 7.5 mmol) in $CHCl_3$ (50 mL) was stirred at room temperature for 1 h. This solution was washed with a 10% Na_2CO_3 solution (3 × 20 mL) and the organic extract was dried and evaporated to obtain a light yellow solid (0.85 g): ^1H NMR ($CDCl_3$, 60 MHz) δ 1.2 (t, 3 H, 8 Hz), 3.1 (s, 3 H), 4.0 (s, 2 H), 4.15 (q, 2 H, 8), 5.85 (s, 2 H), 7.4–8.3 (m, 7 H).

***O*-4-Chloro-2-fluoro-6-nitrophenyl Dimethylthiocarbamate (Cf. 33).** To a solution of 4-chloro-2-fluoro-6-nitrophenol (18.86 g, 0.1 mmol) in DMF (100 mL) cooled to 10 °C was added NaH (4.8 g, 0.11 mol). After 30 min, dimethylthiocarbamoyl

chloride (13.59 g, 0.11 mol) was added to it and the mixture was stirred overnight. The reaction was quenched with ice/water (200 mL) and the resulting crude solid was collected. The solid was dissolved in CH_2Cl_2 (200 mL) and the solution was washed with a dilute $NaHCO_3$ solution and H_2O. The organic layer was dried and evaporated to obtain the title compound (89%); mp 118–119 °C.

***O*-2-Chloro-4-fluoro-6-nitrophenyl dimethylthiocarbamate** was prepared (86%) from 2-chloro-4-fluoro-6-nitrophenol in a similar manner; mp 138 °C.

***S*-4-Chloro-2-fluoro-6-nitrophenyl Dimethylthiocarbamate (Cf. 34).** *O*-4-chloro-2-fluoro-6-nitrophenyl dimethylthiocarbamate (27.8 g, 0.1 mol) was heated at 160 °C for 30 min. After cooling, the residue was crystallized from EtOH (95%); mp 75 °C.

***S*-2-Chloro-4-fluoro-6-nitrophenyl dimethylthiocarbamate** was prepared from *O*-2-chloro-4-fluoro-6-nitrophenyl dimethylthiocarbamate by the above procedure (88%); mp 113–115 °C.

***S*-2-Amino-4-chloro-6-fluorophenyl Dimethylthiocarbamate (Cf. 35).** To a suspension of *S*-4-chloro-2-fluoro-6-nitrophenyl dimethylthiocarbamate (21.0 g, 0.075 mol) in EtOH (150 mL) was added granular tin (26.7 g, 0.225 mol) in small portions. The reaction was exothermic. It was filtered, the filtrate was concentrated, and the concentrate was triturated with H_2O (50 mL) to obtain the title compound as a yellow solid (97%); mp 133 °C.

***S*-2-Amino-6-chloro-4-fluorophenyl dimethylthiocarbamate** was prepared from *S*-2-chloro-4-fluoro-6-nitrophenyl dimethylthiocarbamate in a similar manner (96%); mp 121 °C.

2-Amino-4-chloro-6-fluorobenzenethiol Hydrochloride (129). A solution of *S*-2-amino-4-chloro-6-fluorophenyl dimethylthiocarbamate (16.0 g, 0.065 mol) in ethylene glycol (50 mL) containing KOH (10.8 g, 0.194 mol) was heated at 60 °C, under N_2, for 2.5 h. Excess ethylene glycol was evaporated, the residue was diluted with H_2O (50 mL) and extracted with EtO_2 (2 × 100 mL). The aqueous phase was collected, acidified with HOAc to pH 4.5, and extracted with Et_2O (100 mL). The Et_2O extract was dried over anhydrous $MgSO_4$ and HCl gas was passed into the dried extract. The resulting precipitate was filtered to obtain the desired compound (98%); mp 209–210 °C.

2-Amino-6-chloro-4-fluorobenzenethiol hydrochloride (130) was obtained from *S*-2-amino-6-chloro-4-fluorophenyl dimethylthiocarbamate following the above procedure (86%); mp 208–210 °C.

3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthlazinecarboxylic Acid (48). To a slurry of 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazinecarboxaldehyde[37] (**47**, 20.0 g, 0.051 mol) in acetone (1.0 L) at room temperature was added Jones reagent (3 N, 20.3 mL) dropwise. After 3 h the reaction mixture was diluted with H_2O (1.0 L) and the precipitated solid was filtered. The solid was suspended in NaOH (3 N, 520 mL) and to it was added THF (150 mL). It was then extracted with Et_2O (500 mL). The aqueous layer was collected and acidified with concentrated HCl to pH 2.0. The resulting yellow solid was collected, washed with H_2O (2 × 20 mL), and then air-dried to obtain the title compound (59%); mp 237 °C; ^1H NMR (DMSO, 300 MHz) δ 5.85 (2, 2 H), 7.73 (m, 1 H), 7.95 (m, 2 H), 8.3 (m, 3 H), 8.5 (m, 1 H).

Ethyl 3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazinepropenoate (49a). To a solution of **47** (389 mg, 1.0 mmol) in $CHCl_3$ (10 mL) was added (carbethoxymethylene)triphenylphosphorane (383 mg, 1.1 mmol) and then gently refluxed for 4 h. Excess $CHCl_3$ was removed and the residue was purified by flash chromatography (solvent, 9:1 CH_2Cl_2/EtOAc). The product was a white solid (67%): mp 124–126 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35 (t, 3 H, 7), 4.28 (q, 2 H, 7), 5.88 (s, 2 H), 6.85 (d, 1 H), 7.56 (m, 1 H), 7.9 (m, 4 H), 8.05 (d, 1 H, 18), 8.25 (d, 1 H, 2), 8.5 (m, 1 H).

3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazinepropenoic Acid (49b). A suspension of **49a** (230 mg, 0.5 mmol) in concentrated HCl (4 mL) was heated on a steam bath for 30 min. A heavy white precipitate obtained upon cooling was filtered and the solid was washed with H_2O (2 × 10 mL) and air-dried (91%): mp 227 °C; ^1H NMR (DMSO, 300 MHz) δ 5.88 (s, 2 H), 6.72 (d, 1 H, 15), 7.78 (m, 1 H), 8.0 (m, 3 H), 8.02 (d, 1 H, 15), 8.3 (m, 2 H), 8.4 (m, 1 H).

3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazinepropanoic Acid (49c). To a solution of **49b** (105 mg, 0.25 mmol) in EtOAc (5 mL) was added 10% Pd–C catalyst (10 mg) and hydrogenated in a Parr apparatus at 40 psi for 30 min. The catalyst was filtered off and the filtrate was evaporated to yield the title compound (90%): mp 199–200 °C; ^1H NMR (DMSO, 300 MHz) δ 2.73 (t, 2 H, 9), 3.26 (t, 2 H, 9), 7.78 (m, 1 H), 7.94 (m, 1 H), 8.06 (m, 1 H), 8.33 (m, 3 H).

3,4-Dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetonitrile (51). A mixture of **216** (15.0 g, 35.9 mmol) and acetic anhydride (100 mL) was refluxed overnight. Excess acetic anhydride was removed and the residue was dissolved in Et_2O. The ethereal solution was cooled to around 10 °C and NH_3 gas was bubbled in to obtain a white precipitate (99%): mp 242–245 °C; ^1H NMR (DMSO, 250 MHz) δ 3.8 (s, 2 H), 5.75 (s, 2 H), 7.65 (m, 2 H), 7.8 (m, 3 H), 8.25 (m, 2 H). To the entire product (15.0 g, 36 mmol) dissolved in CH_2Cl_2 (200 mL) was added phosphorus oxychloride (3.3 mL) and then the mixture was refluxed for 6 h. The solution was evaporated to dryness and the residue was quenched with ice/water (200 mL) and then extracted with EtOAc (2 × 100 mL). Evaporation of the organic layer gave the desired product (83%): mp 180 °C; ^1H NMR ($CDCl_3$, 300 MHz), δ 4.1 (s, 2 H), 5.85 (s, 2 H), 7.6 (dd, 1 H, 2 and 8), 7.75–7.95 (m, 4 H), 8.3 (d, 1 H, 2), 8.5 (dd, 1 H, 2 and 8); MS *m/e* 400.0651 (M^+).

4-(1*H*-Tetrazolyl-5-ylmethyl)-2-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1(2*H*)-phthalazinone (52). To a solution of **51** (0.5 g, 1.25 mmol) in DMF (2 mL) was added NH_4Cl (67 mg) and NaN_3 (82 mg, 1.25 mmol) and the mixture was heated at 120 °C for 2 days. It was poured onto H_2O (145 mL), the pH of the solution was adjusted to about 8 and then it was extracted with EtOAc (2 × 10 mL). The aqueous layer was acidified with concentrated HCl to obtain a slightly yellowish solid. The solid was collected and crystallized from MeOH (20%): mp 240 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 3.3 (s, 1 H), 4.6 (s, 2 H), 5.78 (s, 2 H), 7.5 (dd, 1 H, 2 and 8), 7.6–7.9 (m, 4 H), 8.2 (d, 1 H, 2), 8.35 (dd, 1 H, 2 and 8); MS *m/e* 424.0878 ($M^&$).

Molecular Mechanics Calculations. MMP_2 calculations were performed by using Allinger's MMP_2 1980 force field.[38] Calculations were done on a Microvax II computer. Computational times ranged from 4 to 5 min.

Single-Crystal X-ray Analyses. Representation crystals for compounds **216**, **246**, **248**, **254**, and **288** were surveyed and 1-Å data sets (maximum sin $\theta/\lambda = 0.5$) collected on a Nicolet R3M/μ diffractometer. Atomic scattering factors were taken from the *International Tables for X-ray Crystallography*.[39] All crystallographic calculations were facilitated by the SHELXTL[40] systems. All diffractometer data were collected at room temperature by using Cu Kα radiation (λ = 1.54178 Å).

Trial structures were obtained by direct methods. These trial structures refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on nitrogen and oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least-squares refinement were all less than 0.1 of their corresponding standard deviations. A final difference Fourier revealed no missing or misplaced electron density. Crystal and refinement parameters of compounds **216**, **246**, **248**, **254**, and **288** are summarized in Table X.

Biological Methods. Aldose reductase was partially purified from human placetae by a modification of Hayman and Kinoshita's[41] procedure for purification of rat lens aldose reductase. Freshly obtained human placentae were homogenized in 3 volumes

(37) We thank Dr. C. A. Lipinski of our Medicinal Chemistry Department for this procedure.

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Table X. Single Crystal X-ray Data

	216	246	248	254	288
		A. Crystal Parameters			
formula	$C_{19}H_{12}O_3N_3SF_3 \cdot H_2O$ (437.4)	$C_{18}H_{11}O_3N_3SF_2$ (387.4)	$C_{18}H_{11}O_3N_3SCl_2$ (420.3)	$C_{20}H_{11}O_3N_3SF_6 \cdot H_2O$ (487.4)	$C_{17}H_{10}O_3N_4SF_2$ (388.4)
crystallization medium	sodium phosphate buffer	methanol	methanol and chloroform	ethyl acetate	methanol
crystal size, mm	0.15 × 0.19 × 0.19	0.06 × 0.07 × 0.16	0.06 × 0.12 × 0.26	0.10 × 0.26 × 0.30	0.14 × 0.15 × 0.18
cell dimensions					
a, Å	8.823 (2)	8.240 (3)	8.210 (3)	8.505 (3)	8.725 (3)
b, Å	9.269 (2)	24.716 (8)	8.707 (3)	9.155 (3)	17.95 (1)
c, Å	13.242 (3)	11.254 (4)	13.474 (4)	13.531 (4)	10.296 (5)
α, deg	71.65 (2)	90.00	92.15 (3)	87.04 (2)	90.0
β, deg	78.19 (2)	133.17 (2)	90.61 (3)	85.86 (3)	98.95 (3)
τ, deg	70.70 (2)	90.00	115.04 (3)	80.07 (3)	90.0
ν, Å^3	963.9 (4)	1672 (1)	871.7 (5)	1034.2 (6)	1592.86 (1)
space group	$P\bar{1}$	$P2_1/n$	$P\bar{1}$	$P\bar{1}$	$P2_1/n$
molecules/unit cell	2	4	2	2	4
density obsd, g/cm^3	1.49	1.53	none		1.59
density calcd, g/cm^3	1.51	1.54	1.60	1.62	1.62
linear absorption coefficient, cm^{-1}	19.55	21.14	47.5	22.1	22.38
		B. Refinement Parameters			
number of reflections	1985	1711	1782	2134	1642
nonzero reflections ($I > 3.0\sigma$)	1720	1422	1506	1967	1444
R index[a]	0.042	0.054	0.066	0.053	0.042
GOFD[b]	1.91	1.45	1.18	2.16	1.59
scale factor	1.685 (2)	1.746 (3)	1.517 (4)	1.679 (3)	1.679 (3)
secondary extinction coefficient	7 (1) × 10^{-3}	33 (7) × 10^{-4}	18 (3) × 10^{-3}	6 (2) × 10^{-4}	32 (1) × 10^{-4}

[a] R index = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] GOF = $[\sum w(F_o^2 - F_c^2)/(m-s)]^{1/2}$ where $w = [\alpha^2(F) + |g|F^2]^{-1}g = 0.0010$.

of 0.1 M potassium phosphate buffer, pH 7.0, containing 5 mM 2-mercaptoethanol and centrifuged for 20 min at 33000g at 4 °C. The supernatant was subjected to a 50%–75% ammonium sulfate fractionation and the resulting pellets were pooled, resuspended in a minimum volume of homogenization buffer, and dialyzed overnight against the same. The dialysate was applied to a DEAE-cellulose column (2 cm × 25 cm) and aldose reductase was eluted with a linear salt gradient (0–1 M NaCl) in buffer. Peak fractions containing aldose reductase activity were pooled and aliquots were stored frozen.

Enzyme activity was assayed with an Abbott VP bi-chromatic clinical analyzer which measured the decrease in the rate of NADPH oxidation at 340 nm at 25 °C over 12 min in a reaction mixture of 0.25 mL of 50 mM potassium phosphate buffer (pH 7.1) containing 0.4 M ammonium sulfate, 0.067 mM NADPH, and 1.0 mM *dl*-glyceraldehyde. Sufficient enzyme was added to produce a rate of NADPH oxidation equal to 4 milliunits (unit equal to 1 μmol of NADPH oxidized at 25 °C per min). IC_{50}s were calculated by using a log linear-regression analysis.

Acute in vivo evaluation was conducted as follows. Rats (n = 4) were made diabetic by a single iv injection of streptozotocin (86 mg/kg). The inhibitor was then administered by oral gavage at the indicated doses at 4, 7, and 24 h. At 27 h the animals were sacrificed and the sciatic nerve and lens were removed for sorbitol determination. Inhibition is calculated on the basis of comparison to untreated diabetic animals (n = 4) and significance was calculated by using Student's t test ($p < 0.05$). In dose titration studies ED_{50}s were calculated by using a log linear-regression analysis.

Chronic in vivo evaluation was performed as follows. Rats (n = 5) were made diabetic by iv injection of streptozotocin (85 mg/kg) and left untreated for 7 days. The inhibitor was then administered once daily by oral gavage at the indicated doses for the next 5 days. Four hours after the last dose the rats were sacrificed and tissues were removed for sorbitol determination. Inhibition was calculated on the basis of comparison to untreated diabetic controls and significance was calculated by using Student's t test ($p < 0.05$). None of the compounds had any effect on the glycemic state of the animals in either of the in vivo tests.

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Supplementary Material Available: A listing of atomic coordinates, isotropic and anisotropic thermal parameters, bond lengths and angles, and H atom coordinates for compounds **216**, **246**, **248**, **254**, and **288** (24 pages). Ordering information is given on any current masthead page.